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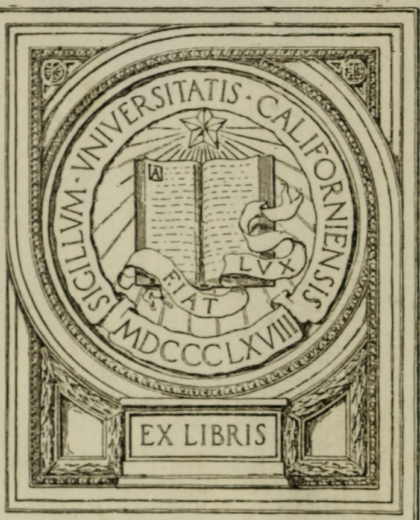

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EXPERIMENTAL PHARMACOLOGY

GREENE

EXPERIMENTAL PHARMACOLOGY

A LABORATORY GUIDE

FOR THE STUDY OF

THE PHYSIOLOGICAL ACTION OF DRUGS

BY

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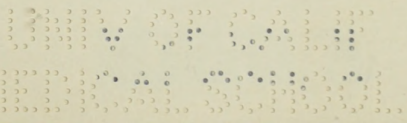

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PREFACE.

Instruction in Pharmacology should be based on a rigid course of required laboratory experiments. The student in the subject must be given every opportunity to observe for himself the changes produced by a drug in the activities of a tissue, of an organ, and of the entire organism. It is only on such intimate personal experience with the facts that one can reach a rational understanding of the principles of Pharmacology.

The directions presented here have been formulated during the growth of the course as presented in the University of Missouri. Under each drug presented there is given a list of experiments chosen with due consideration to the facility with which they may be performed by students. This list is followed by detailed yet brief directions for the execution of the experiments. An occasional type illustration is given to guide the student in his efforts. It is assumed that the individual student will have time for only a selected number of experiments on each drug, and the plan of the Guide is so arranged as to support the instructor in the assignment and execution of a number of diversified experiments by the average laboratory class.

I am indebted to the Department Teaching Staff, especially to Professor W. Koch, now of the University of Chicago, to Dr. W. H. Schultz, now of the U. S. Public Health and Marine Hospital Service, and to Professor R. B. Gibson for numerous suggestions and much assistance.

I am under special obligation to Mr. G. T. Kline for the drawings of apparatus, to Professor Gibson and to my students of the past five years for the majority of the new illustrations presented in this edition of the Guide.

C. W. G.

University of Missouri, *April*, 1909.

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EXPERIMENTAL PHARMACOLOGY

THE ACTION OF DRUGS.

ALCOHOL.

List of Experiments Showing the Effects of Alcohol.

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1. Alcohol on the frog. Inject into the dorsal lymph sacs of two frogs doses of **0.3 c.c.** (5 minims) and **0.6 c.c.**, respectively, of **95 percent alcohol.**[1] Strong alcohol is quickly absorbed from the lymph sac. The larger dose is sufficient to produce temporary complete loss of the reflexes, together with the loss of all respiratory movements. A dose of **1 c.c.** is toxic for a 40-gram frog. Since the smaller dose is equivalent to **525 c.c.** for a 70-kilo man, it is evident that the frog is the more tolerant of alcohol.[2]

2. Alcohol on ventricular muscle. Mount a strip of the ventricle of a terrapin in 0.7 percent sodium chloride (see page 66 for the method), and when it is contracting with an even and regular rhythm change to a solution of **2 percent alcohol** in physiological saline. Return the strip to

[1] All doses for frogs given in this book are calculated for an animal weighing 40 grams. Each animal used for experiment should be weighed on a "Harvard" platform balance and the dose given calculated in proportion to the weight of the animal used.

[2] A report blank form for the tabulation of observations on the effect of drugs on frogs is given on page 76.

pure saline solution after two to five minutes. Record the contractions on a drum moving 1 mm. a second. Repeat the experiment, using successive strengths of alcohol of **5 and 10 percent.** The alcoholic effect will be demonstrated rather better on a ventricular strip that is contracting regularly in the weaker Ringer's solution, page 64, but the alcohol must be dissolved in Ringer's solution of the same composition.

3. Alcohol on the frog's heart. Destroy the brain and spinal cord of a frog, expose the heart by cutting away the ventral wall from directly over the ventricle, using care not to lose blood. Do not cut the bridge formed

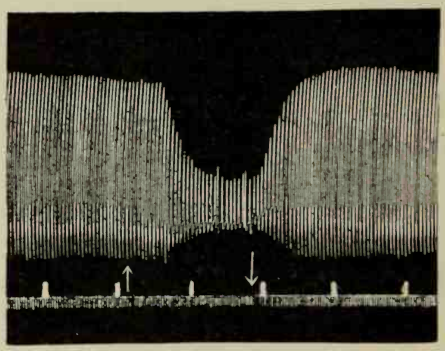

FIG. 1.—Action of 2 percent alcohol on the frog's heart when perfused through the ascending vena cava. The alcohol was dissolved in Ringer's solution. The record was taken from the tip of the ventricle by the suspension method. Time in seconds and minutes. Perfusion began at the first arrow above the second's record and ended at the second arrow.

by the sternum, but use it as a fixed point to which the heart may be anchored by a ligature around one of the aortic arches. Take a direct record of the movements of the ventricle, using a light straw lever of the fulcrum-power-weight order. Give **1.5 c.c. of 95 percent alcohol** in the abdominal cavity. Take a continuous record during the time of absorption. This method demonstrates the effects on the volume, and on the type of systole and diastole. The rate is only slightly changed.

More satisfactory results are obtained by perfusing the heart through a canula in the ascending vena cava. Perfuse the alcohol from four-ounce supply flasks provided with constant level tubes. Use a pressure of from 4 to 6 cm. The perfusion strength to use by this method is **2 to 5 percent alcohol** made up in Ringer's solution. Perfuse the heart for from two to

four minutes at a time. Record the contractions of the ventricle by a thread from its tip to the vertical arm of a balanced lever, page 68. In this experiment, as in all frog's heart perfusion, it is better to use the weaker Ringer for the normal solution. The Ringer's solution insures a uniform heart rate and strength for long intervals, while the sodium chloride solution will sustain the whole heart in regular and strong rhythm for only a few minutes.

4. Alcohol on the isolated mammalian heart. Use a rabbit or a cat for this experiment. Anesthetize quickly with ether (do not use chloroform), insert a canula in the carotid, bleed completely, defibrinate the blood and dilute it with nine volumes of Locke's solution. Use this diluted blood as a normal solution for perfusing the heart. Reserve enough of the solution for making the drug mixtures, pour the remainder into the perfusion apparatus described on page 73, fill the tubes, adjust the apparatus and bring to a constant temperature of 36 to 37° C. (a higher temperature is unfavorable). Quickly remove the heart, taking care only to preserve enough of the aorta for the insertion of the canula without danger of interfering with the semilunar valves. Mount the heart without catching air in the canula, attach the recording lever of the Guthrie cardiograph and start the perfusion. The perfusion pressure should be from 80 to 100 cm. of water.

The heart contracts and a uniform rhythm will be quickly established and may be maintained for several hours. Perfuse the heart with **0.2 percent alcohol in the Locke-blood solution** for from 30 to 100 seconds at a time, allowing full time for a return to the normal after each drug perfusion. Raise the dose successively to **0.4 percent, 1 percent,** and **2 percent of alcohol.** The stronger solutions reduce the amplitude and ultimately the rate of the heart; the weaker doses, according to Dixon, increase the amplitude.

5. Alcohol on muscular work. Ligate one leg of a frog near the thigh to exclude its circulation (a quarter-inch rubber tube makes a fine ligature for this purpose). Inject **0.3 c.c.** (5 minims) **of 95 percent alcohol** into the dorsal lymph sac. In exactly twenty minutes pith the frog, pin it out on the frog-board with the ventral side down, and ligate the alcoholized leg. Quickly prepare the tendon of the normal muscle, keeping the muscle covered with skin, attach to the muscle lever, and determine the work it can do by stimulating the muscle directly with a single induction shock once every two seconds until the muscle is completely exhausted. Load the muscle with a 30- to 50- gram weight. Record the contractions on the lower half of a drum with a speed of 1 mm. a second.

Prepare the second or alcoholized muscle just twenty minutes after it has been ligated, mount, load, and stimulate in the same manner. Record the second experiment on the upper half of the same smoked paper and parallel with the record of the first. Repeat this experiment using a dose of **1 c.c. of 95 percent alcohol,** to demonstrate the injurious effects of alcohol on the work of the muscle. (Lee and Salant, Am. Jour. Physiology, Volume VIII, p. 61, 1902.)

A more difficult but more accurate experiment is obtained as follows: Destroying the brain only of a frog, pin it to the frog-board belly down, dissect out the right tendon Achilles, and attach to a muscle lever. Isolate the lumbar plexus on the right of the urostyle, using care not to interfere with the circulation of the gastrocnemius. Stimulate the right muscle as above. Now inject alcohol into the abdominal cavity, and after 20 to 30 minutes of absorption measure the work of the left gastrocnemius. This method has the advantage of maintaining the circulation intact for both the normal and the alcoholized muscle.

The effect of alcohol on the speed of the contraction can be determined by one of two methods. One can measure the simple muscle contraction before and after alcoholization. Perhaps a better method is to use Lee's automatic stimulating device (Am. Jour. Physiol., VIII, p. 61) which automatically stimulates a muscle at the moment of complete relaxation. Record the contractions on a slowly moving drum, 2 mm. per second.

6. Alcohol on voluntary work of human muscle. Demonstration. Measure the voluntary power of the flexors of the middle finger with a load of three kilos or more, using Mosso's ergograph. Take two or three normal records of voluntary contractions at intervals of 20 minutes. Now take a dose of **20 to 40 c.c. of 20 percent alcohol,** according to the susceptibility of the individual. Remeasure the muscular power after 60, 90, and 120 minutes, respectively. Compute the work done in kilogrammeters. (Lombard, Jour. Physiol., Vol. 13, p. 49; Hallsten, Skand. Arch. f. Physiol. Bd. 16. S. 139.)

7. Alcohol on the circulatory and respiratory systems of the mammal. Anesthetize a dog with morphine and chloroform, p. 70. Take the blood-pressure from the carotid artery, and the respiration from a side branch of a tracheal canula. Expose the saphenous vein and insert a canula for intravenous injections, and attach it to a 50 c.c. buret.

Students who have attained the requisite skill should take an onkometric record with the blood-pressure. To prepare for this record open the abdominal cavity of the dog, remove the outer sheath from the left kidney

and enclose that organ in a renal onkometer. Record the kidney volume changes by means of a Brodie's bellows, or Roy's piston recorder, page 71.

The anesthetic must be given with perfect regularity, 2 to 6 drops of chloroform every 30 seconds, the exact amount that will maintain constant anesthesia to be quickly ascertained for each animal.

Take a record on the continuous kymograph and, when all is in good working condition and a normal record has been secured, slowly inject **20 percent warmed alcohol** from the buret into the vein until some decided effect on the blood-pressure is noted, i.e., after a dose of **20 c.c. or more.** Extreme caution must be observed lest the heart by rapid perfusion be subjected to an overconcentrated solution. The experiment should be repeated with different doses.

Since the anesthetics used all depress the irritability of the circulatory apparatus, this experiment ought to be demonstrated on a decerebrate animal. In such an animal the medulla being intact will maintain natural respirations. Any alcoholic stimulation of the medullary centers can easily be observed. The recommended intravenous dose of alcohol will produce slowing of the heart, a phenomenon which disappears on section of the vagi, thus indicating a direct effect on the vagal centers.

8. Alcohol on the reaction time of the reflex frog. Destroy the brain of a frog, including the medulla, and when it has recovered from the shock test the normal reaction time to electrical stimuli applied to the toe. Measure the time of the reaction with a watch, or record it with a writing-point attached to the foot or leg of the suspended frog. Give a dose of **0.3 c.c.** (5 minims) **of 95 percent alcohol** in the dorsal lymph sac. Retest the reaction time at exactly 20 and 40 minutes after the injection. Compare the results with experiments 1 and 5 above.

ETHER.

List of Experiments Illustrating the Effects of Ether.

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8. On the germination of seeds 10
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1. **Ether on the frog.** Inject **0.2 c.c.** (3 1/2 minims) **of ether**[1] into the dorsal lymph sac or the abdominal cavity of a frog. Give **0.3 c.c.** to a second frog. The dose can be given more accurately from the hypodermic if a 50 percent solution of ether in olive oil is used. The first dose will produce anesthesia in about 10 minutes. The stages most readily observed are: 1st, great excitement shown by rapid respirations, active movements, and increased reflex irritability; 2d, slower respirations, very sluggish response to external stimulation; 3d, loss of voluntary muscular control and sometimes of respiratory motions. Slight power of reflex response is retained, including eye reflexes. The voluntary motions will be regained in from 60 to 90 minutes if the animal is kept moist (winter frogs), and complete recovery in two hours. The frog will recover from the larger dose in from 20 to 24 hours, or it may even fail of recovery.

2. **Ether on the ventricular muscle.** Mount a strip of terrapin's ventricle and establish rhythmic contractions in a bath of 0.7 percent saline. Record on a drum moving 1 to 2 mm. per second. Immerse the strip in a bath of **1 percent ether** in saline for two to three minutes, then return to the physiological saline bath. The sharp decrease in both amplitude and rate of contractions is recovered quickly in the saline bath.

Repeat the experiment using **2, 4, and 6 percent ether solutions.** The weaker solutions occasionally produce slight but temporary increase in the rate, the initial excitation stage. Also use strips of auricle and sinus.

[1]The dose is figured for a 40-gram frog. Proportionate doses should be given for other weights. In all experiments on frogs that depress their functions, the animal should be retained in a moist bell jar for as much as 24 hours, if necessary, in order to test the animal's power of recovery.

3. Ether on the frog's heart. Pith a frog, expose its heart, insert a canula in the inferior vena cava and perfuse the heart in place by the method described for alcohol, experiment 3. This brings the solution into intimate contact with the entire heart and it responds almost instantly to any change in the composition of the irrigating fluid. Perfuse the heart first with Ringer's solution and follow with **1 percent ether in Ringer's solution.**

4. Ether on the mammalian heart. Use the Roy-Adami method (given by Cushny, Jour. Exp. Medicine, Volume II, page 233) or the

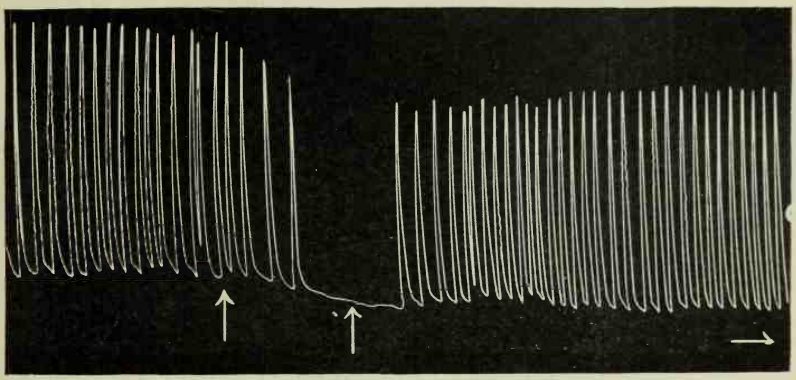

FIG. 2.—The action of ether on isolated heart muscle. In this experiment a strip of terrapin's ventricle was mounted in physiological saline until a regular rhythmic beat was established. The saline was then drawn off and the strip left suspended in moist air. At the point indicated ether vapor was driven through the moist chamber until the contractions ceased. The ether vapor was then removed with moist air at the second arrow and the recovery of the rhythm took place as shown.

method described on page 73 and used in experiment 4 of the alcohol series. Etherize a cat or rabbit, draw the blood, defibrinate it, and dilute it with nine volumes of Locke's solution and use as a standard Locke-Blood perfusion fluid. Remove the heart and adjust it in the apparatus, page 74. Perfuse with the Locke-Blood solution and when the rhythm is established change to **1 percent ether in Locke-Blood.** Use **2 percent ether** in a later experiment.

5. Ether on the irritability of voluntary muscle. Mount a gastrocnemius of the frog in the moist chamber, arrange to stimulate the muscle directly with a current of medium intensity but which produces a maximal contraction. Adjust a vapor apparatus containing **saturated**

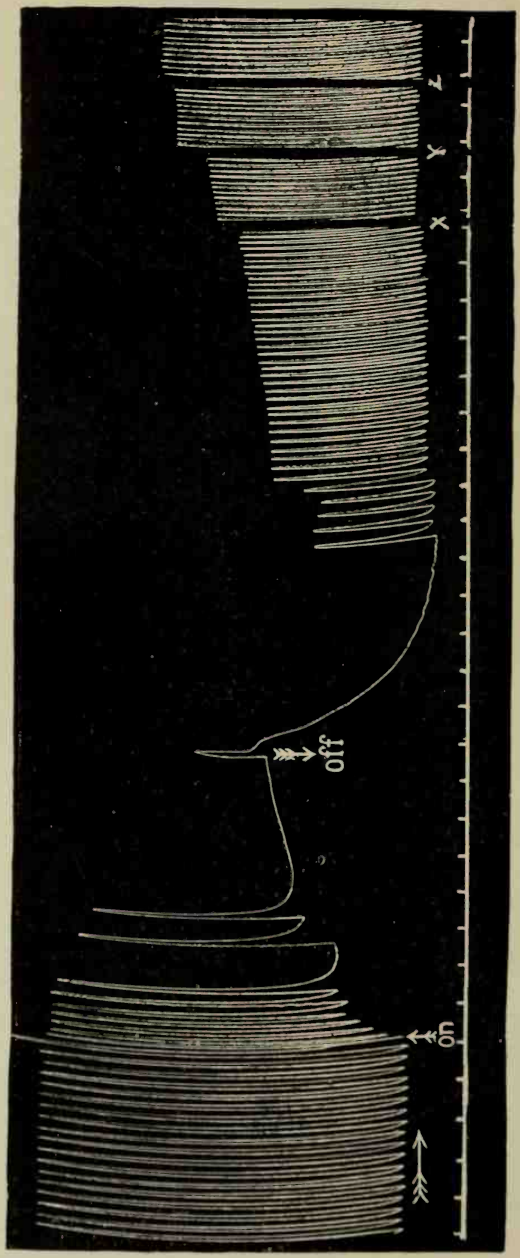

FIG. 3.—Effect of strong ether solution upon the ventricle muscle of the terrapin. Six percent ether in physiological saline was used, with saline for a normal solution. At X, Y, and Z three-minute intervals occur. Time in 30-second intervals

ether water ready for quick connection with the gas tube of the moist chamber. Stimulate the muscle with single induction currents once every 30 seconds throughout the entire experiment, whether contractions are secured each time or not. Record on a drum having a rate of 2 mm. per 10 seconds.

Take records, three or four normal contractions, then turn on the **ether vapor** for five minutes. Quickly remove the vapor with a current of fresh air. The muscle's irritability will decrease to a point at which the stimulus is submaximal or even subminimal, but when the ether vapor is removed the contractions quickly reappear and attain their former amplitude. Use small muscles for this experiment.

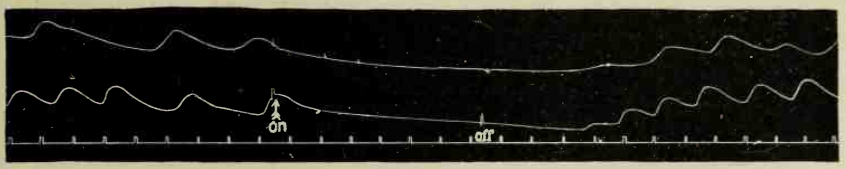

FIG. 4.—The action of ether on isolated strips from the auricle (upper trace) and from the sinus (lower) of the terrapin. The strips were giving tone contraction waves, but no fundamental rhythm when bathed with normal sodium chloride solution. At the mark "on" the sodium chloride was changed to **one percent ether** in normal saline for seven minutes, then again to the normal. During the ether bath the tone waves disappear, a condition which lasts for three minutes after the ether is removed. Following the ether there is a renewal of the tone waves which are even more rapid than in the normal. Time in minutes.

One may with this preparation also demonstrate that the muscle has a diminished power to do work when etherized, method page 72.

6. Ether on the irritability of nerve tissue. Prepare a muscle nerve of the frog, isolating the entire sciatic with a piece of cord, and with the skin covering the muscle. Mount the preparation with the nerve in the moist chamber and on the electrodes, but with the muscle hanging through the hole in the floor of the moist chamber and on the outside so that it will not be etherized. Close the hole with a sheet of moist filter-paper. Proceed exactly as in experiment 5 above testing the irritability of the nerve through its effect on the muscle.

The influence of ether on nerve irritability may also be demonstrated directly from the nerve by the action current method. For a description of the method, see Am. Jour. Physiol., Volume I, p. 104.

7. Ether on the blood-pressure and on the respiration of the mammal. Anesthetize a dog with morphine and ether. Introduce an

arterial canula in the carotid for taking the blood-pressure record, see page 70 for details of method. Insert a tracheal canula and take a record of intra-tracheal pressure from a T-tube attached to the canula. Give ether from an ether bottle connected to the end of the tracheal tube. Take a continuous record and ultimately give excess of **ether,** then allow partial and guarded recovery. Give ether to the point where respirations cease, a point attained with difficulty except when the animal has a large dose of morphine. The blood-pressure is an index of safety, for it has been shown that respiratory impulses are quickly re-established when the blood-pressure remains high.

Saturated ether in saline as an intravenous injection in doses of **20 c.c.** and more, given along with a uniform administration of ether by the trachea,

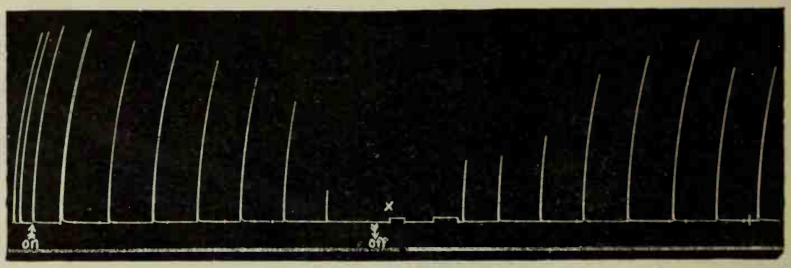

FIG. 5.—The influence of ether vapor upon the irritability of the sciatic nerve in the muscle-nerve preparation of a frog. The muscle-nerve preparation was suspended in a moist chamber with the muscle hanging through the opening in the chamber. The first two contractions are normal. The next eight are successive contractions at intervals of ten seconds during the passing of ether vapor. At the **X** the ether vapor was removed by a stream of moist air, the nerve being stimulated at intervals of ten seconds until recovery of the irritability as shown by the contractions at the last part of the experiment. A gap of two minutes occurs at X, during which the nerve was not irritable. Time in seconds.

will often demonstrate the characteristic circulatory and respiratory effects of the drug.

The rectal temperature should be recorded at intervals to demonstrate the fall of temperature under anesthetics. Note also the state of dilatation of the pupil.

8. Ether on the germination of seeds. Arrange two eight-ounce wide-mouth bottles with stoppers each fitted with two glass tubes, letting one tube extend to near the bottom of the bottle. Suspend in each, by means of cotton, a dozen seeds—corn, wheat, clover, beans, etc.—and introduce just enough water to maintain a saturated vapor. Set both bottles in a window. Twice a day for a week, pass through one saturated **ether**

vapor, through the other **air.** The seeds in both will swell from the absorption of the water, but only the seeds in the bottle with pure air introduced will grow. Reverse the two. The sprouting grain will have its growth checked and the etherized seeds will begin to grow.

9. Ether on the yeast. Take two fermentation tubes of active yeast culture, add 2 c.c. pure ether to one. Note the relative rate of gas liberation.

CHLOROFORM.

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1. Chloroform on the frog. Inject **0.06 c.c. of pure chloroform or 0.3 c.c. of 20 percent chloroform** in olive oil into the dorsal lymph sac or into the abdominal cavity of a frog. The anesthesia is more profound and the recovery less rapid than in the case of ether. Determine the relative intensity of action of chloroform and ether by your own experiments.

2. Chloroform on the heart strip. Proceed as with ether in experiment 2, page 6, using a **0.05 percent** solution of chloroform in 0.7 percent saline for one to three minutes. The contractions cease almost at once. Recovery in saline takes place very slowly. In comparison with ether the period of anesthesia is long. The amplitude of the first contractions to reappear is very slight and the recovery rate slow and irregular. The original character of activity is not restored within 20 to 40 minutes. Repeat using a solution of **0.1 percent chloroform.**

An instructive picture is given by parallel records of experiments on strips from the same heart showing the effects of **4 percent ether** and of **0.1 percent chloroform** for two minutes, both in saline.

3. Chloroform on the frog's heart. Proceed as in the similar experiment with ether, page 7, using **0.5 percent chloroform in physiological saline** to irrigate the outer surface of the heart. Or perfuse the heart

FIG. 6.—Action of chloroform vapor upon the rhythm of isolated heart muscle. A strip of terrapin's ventricle contracting in the moist chamber was exposed to chloroform vapor at the point indicated. The chloroform vapor was removed with a stream of moist air. The recovery of rhythm and amplitude of contractions in this experiment is to be compared with that shown in Figures 2 to 4.

with **0.05 percent chloroform** in saline through the vena cava. Care must be used not to prolong the action of the drug. The amplitude is reduced to one-half and the rate markedly slowed or entirely suppressed. Both rate and amplitude are recovered, but not so quickly as with ether.

4. Chloroform on the mammalian heart. Determine the action of chloroform on the isolated cat's heart, using the method described for the alcohol experiment 4, page 3. Perfuse the heart with **chloroform 0.02 to 0.05 percent** in the Locke-blood. The chloroform perfusion must be for short periods and be guarded closely.

5. Chloroform on the muscular irritability. See ether experiment 5, page 3. Use **0.1 percent chloroform water** in the vapor apparatus. Care must be taken to remove the saturated chloroform vapor from the vapor apparatus just before using, otherwise the muscle, or the nerve in the next experiment, will be over anesthetized and will not recover its irritability. There is no danger with ether from this cause.

6. Chloroform on the irritability of the nerve. Repeat ether experiment 6. Use **0.1 percent chloroform water** in the vapor apparatus.

7. Chloroform on the blood-pressure and on the respiration of a mammal. Proceed as with ether experiment 7, page 4, using chloroform to anesthetize the dog or cat (rabbits are too sensitive to chloroform for use in this experiment except in practiced hands). Remember that chloroform is said to be about forty times as strong as ether in its

general effects on the animal body. If the vagi are intact and the animal is anesthetized without tracheotomy there will be marked slowing of the heart rate together with a sharp fall of blood-pressure. This effect is

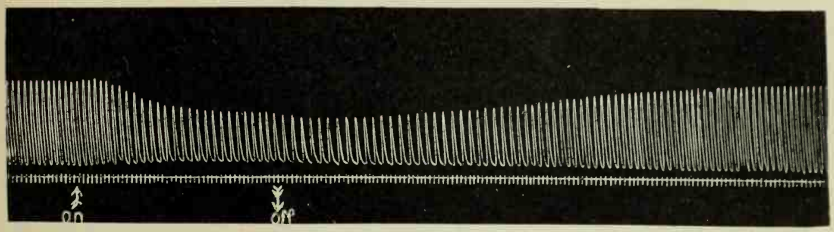

FIG. 7.—Chloroform perfusion of the frog's heart. Ringer's solution used for the normal, **0.1 percent chloroform** between "on" and "off." Perfusion pressure 4 cm. Record from the suspended apex. Time in seconds.

eliminated by section of the vagi. With extreme care chloroform anesthesia may be pushed to the point where respirations cease, and the animal be recovered without artificial respiration. Often, however, in 5 to 10 seconds

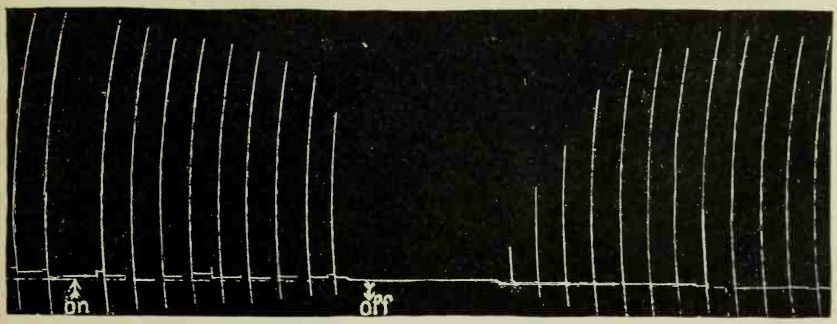

FIG. 8.—The effect of chloroform on the irritability of the nerve in the muscle-nerve preparation. The first two contractions represent the normal amplitude upon stimulating the nerve of a preparation mounted in a moist chamber. The muscle was allowed to hang through the opening in the floor of the moist chamber, so as to protect it from chloroform vapor. At the point indicated by the word "on" chloroform vapor was driven through the moist chamber. The nerve was stimulated at regular intervals until no further contractions occurred. The chloroform was next removed with moist air and the stimulations continued. After a short interval contractions gradually resumed until they reached their normal maximum.

after respirations cease, the blood-pressure will suddenly sink to a low level, and the heart will become weak and slow (see experiment 8 below),

a state from which recovery can be secured only by rapid and vigorous artificial respiration.

Give chloroform intravenously in doses of **10 to 20 c.c. of 0.5 percent** solution in saline, allowing plenty of time for recovery in each test. Compare with alcohol and ether.

8. Chloroform on the secretion of the kidney. Anesthetize a dog with morphine, 1 c.c. of 1 percent, and chloroform, avoiding deep anesthesia during the preliminary preparations. Insert a ureter canula and connect it with a horizontal glass tube mounted on a graduated scale, or see methods, page 70. Take a continuous record of the arterial pressure.

Now determine the normal rate of secretion of urine per 10 minutes for at least 40 minutes, keeping the dog under light but constant anesthesia. Inject intravenously **10 c.c. of 0.5 percent chloroform** solution in saline. Double the dose if necessary until profound anesthesia with low blood-pressure and weak heart is obtained. Or produce deep anesthesia by means of the respiratory inhalations. Recover and maintain light anesthesia for an hour or more. The circulation is quickly re-established in good condition, but the secretion of urine which is suppressed during the stage of deep anesthesia is more slowly brought up to the normal with the re-establishment of good circulation.

9. Chloroform on germinating seeds. Repeat the experiment described for ether, page 11, passing air from saturated chloroform water into one bottle of seed, and pure air into the other. After the seeds in air have sprouted, reverse the bottles. Both seeds and young growing plants are anesthetized by chloroform. The seeds may not grow later, as the drug kills plant protoplasm when given beyond a rather narrow limit of both time and concentration.

CHLORAL HYDRATE.

Experiments Showing the Effects of Chloral Hydrate.

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1. On the frog. 14
2. On the rabbit or cat. 15
3. On the heart of the frog. 15
4. On the heart muscle. 15

1. Chloral hydrate on the frog. Give a hypodermic injection of **0.5 c.c. of 2 percent chloral hydrate** dissolved in **0.7 percent** saline. Keep the animal in a moist battery jar until complete recovery. Give particular attention to the effects on the circulatory and the nervous systems.

2. Chloral on the rabbit or cat. Give a hypodermic injection of **2 c.c. of 2 percent chloral hydrate** per kilo of body weight in saline. Repeat in sixty minutes if necessary to produce the chloral narcosis. Make close comparison with the effect of morphine and strychnine.

3. Chloral hydrate on the frog's heart. Pith a frog and take tracings of the ventricle when irrigated over the surface with **1 percent chloral hydrate.** Or perfuse the heart with **0.2 percent chloral hydrate** in Ringer's solution and take tracings of the ventricle by the method described on page 69. Note that the recovery period is unusually long.

4. Chloral hydrate on heart muscle. Prepare a ventricular strip of the terrapin and establish contraction in a bath of **0.7 percent sodium chloride** as usual. Change to a bath of **0.1 percent chloral hydrate** in sodium chloride. Stronger solutions may suppress the rhythm entirely.

THE OPIUM SERIES.

Experiments Illustrating the Effects of Morphine, Codeine and Thebaine.

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2. On the mammal. 15
3. On ventricular muscle. 16
4. On the frog's heart. 18
5. On the mammalian heart. 18
6. On the reflex reaction time. 18
7. On the volume of air breathed. 18
8. The morphine group on the circulation and respiration in the mammal. 18

1. Morphine on the frog. Give a dose of **1 c.c. of 2 percent morphine acetate** in physiological saline in the dorsal lymph sac. Keep the animal under observation for two or more hours to secure the later effects in the frog. An instructive comparison is had by giving a dose of **0.5 c.c.** (8 minims) of **0.1 percent strychnine nitrate** to a second frog at the same time. Keep the frog under observation until complete recovery.

Give a second frog an injection of **1 c.c. of 1 percent codeine** in physiological saline. Compare with morphine.

The dose of **thebaine** for the frog is **1 c.c. of 1 percent.**

2. Morphine on the mammal. Give a hypodermic injection of **1.5 c.c. of 2 percent morphine** under the skin of the shoulder, see anes-

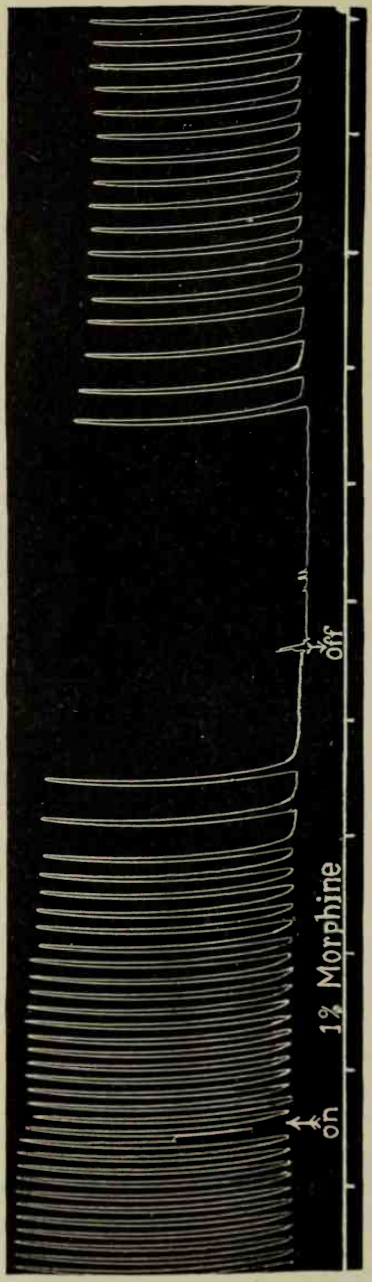

FIG. 9.—Morphine effect on the strip of terrapin's ventricle. The strip was contracting in physiological saline when it was transferred to one percent morphine between the marks "on" and "off." Time in seconds and minutes.

thesia, p. 65. Keep the animal under observation for at least two hours and note at intervals the temperature, irritability, respiratory, ocular, and other changes under the influences of the drug. If vomiting is produced a concentrated watery extract of the vomit will show the presence of morphine, thus demonstrating that excretion of morphine takes place into the stomach.

3. Morphine on the ventricular muscle. Prepare a strip of the ventricle of a terrapin and get it into regular contractions by a bath of 0.7 percent sodium chloride. Change the strip to a bath of **1 percent morphine** in sodium chloride for five minutes, or until a marked change in the rate and amplitude of the contractions occurs, after which the strip should be returned to saline. Record the contraction on a smoked drum moving with a speed of 1 to 2 mm. a second. This experiment shows that the contractions of cardiac muscle are weakened and slowed under morphine. Repeat, varying the conditions according to the results of the previous experiment. A stronger solution of morphine will inhibit all heart muscle activity for many minutes or even hours. The proper strength of **codeine** for this

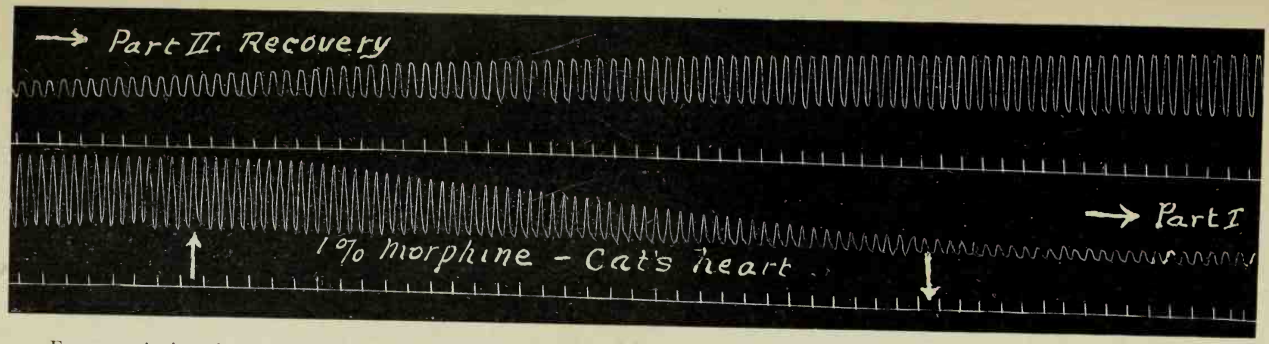

FIG. 10.—Action of morphine on the isolated heart of the cat. The normal perfusion fluid was Locke-blood solution. One percent morphine was perfused between the arrows. This figure shows the usual type of action but occasionally a preliminary stimulation occurs as shown in Fig. 11. Time in seconds. Temperature 34° C.

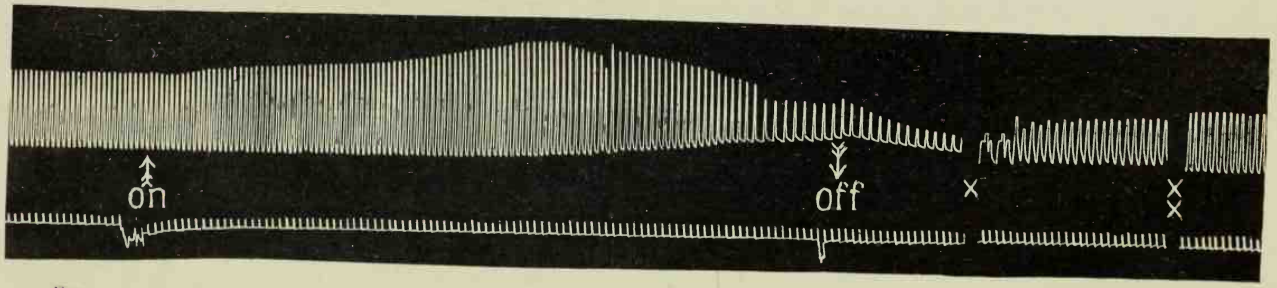

FIG. 11.—Action of morphine on the isolated heart of the cat. The normal perfusion fluid was Locke-blood mixture. Between the points marked a 1 percent morphine in Locke-blood solution was perfused through the aorta and coronary arteries. An interval of one minute occurs at X, and of two minutes at XX. Time in seconds.

experiment is **0.5 percent** in physiological saline, of **thebaine** about **1 percent solution.**

4. Morphine on the frog's heart. Pith a frog, expose the heart and take a record of its contractions, either by the perfusion or by the suspension method. Test the irritability of the vagus trunk. If the circulation is still effective give a lymph sac injection of **0.5 c.c. of 10 percent morphine acetate** or apply drops of this solution directly to the heart from the dropping bottle. Retest the effectiveness of the vagus stimulation. If the heart is perfused, the effective solution to use is **0.5 percent of morphine acetate** in Ringer's solution.

5. Morphine on the mammalian heart.—Etherize a cat, collect its blood and isolate its heart as described in alcohol experiment 4. Fill the tubes of the mammalian heart perfusion apparatus, bring the apparatus to a temperature of 36° C., adjust the heart in it and begin the normal perfusion. When the heart is contracting with a regular and relatively even rhythm perfuse it for two minutes with **0.5 percent morphine** in Locke-blood solution. Repeat the experiment using **1 percent** solution. If the perfusion is too prolonged the heart depression will be removed only with the greatest difficulty.

6. Morphine on the reflex reaction time. Test the reaction time in a reflex frog in the usual way, see methods page 71, first on the normal animal, then 20 and 40 minutes after a dose of **1 c.c. of 4 percent morphine acetate.**

7. Morphine on the volume of air breathed. Anesthetize a rabbit (or cat) with two grams urethane and ether, administering the latter at perfectly regular intervals and with a constant number of drops. Keep a continuous record of the respiration rate per minute either by counting or by recording on a smoked drum. Measure the respiration volume as follows: Insert a tracheal canula and connect it with an apparatus for measuring the volume of expired air. Ether can be given through the open tube of the apparatus except when actually measuring the air volume. Fill the graduated cylinder with water. Then measure the volume of eight or ten expirations, according to the volume of the apparatus used, and repeat several times to secure reliable averages of the expiratory volume of the etherized animal. Compute the expiratory volume per minute. Now give **1 c.c. of 2 percent morphine** hypodermic. Remeasure the respiratory rate and expiration volume at intervals of 10 minutes.

8. The morphine group on the circulation and on respiration in the mammal. Anesthetize a dog with chloroform (no morphine), take the

blood-pressure from the carotid, insert a tracheal canula and take the respiration by the intratracheal method. Insert a canula into the saphenous vein and connect with a buret for intravenous injections. (One may readily insert a canula into the ureter and follow the secretion of urine under the morphine. Consult the instructor.)

Give an intravenous injection of **2 c.c. of 2 percent morphine** in saline. The injected solution should be about the temperature of the body. Repeat after ten minutes, using **4 c.c.** When equilibrium is reestablished give **2 c.c. of 1 percent codeine.** Give **2 c.c. of 1 percent thebaine.** Give thebaine first if there is an opportunity to make the test on a second animal. Now cut the vagus nerves and repeat the dose of **2 c.c. of 2 percent morphine.**

The anesthetic must be gradually diminished according to the amount of morphine, etc., that has been injected. Use artificial respiration if necessary.

CAFFEINE.

Experiments Illustrating the Effects of Caffeine.

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6. On the reaction time in man 22
7. On the reflex reaction time in the frog. 22
8. On the mammalian heart 23
9. On the circulation and respiration in the mammal . . . 23
10. As a diuretic 24

1. **Caffeine on the frog.** The dose is 1 c.c. of 0.5 percent caffeine in the dorsal lymph sac. There is usually a great increase in the irritability together with muscular cramps in the later stages, and finally paralysis. There may be considerable opisthotonus from the direct muscular effects at the area of injection. Note the recovery stages when the frog is kept in a moist battery jar.

2. **Caffeine on the ventricular muscle.** Record the contractions of a strip of terrapin's ventricle beating in physiological saline on a slow-speed drum. Change the strip from saline to 0.1 percent caffeine in saline for five minutes or less, then back to saline until the contractions become uniform and typical of the saline curve. Repeat, varying the time of the immersion in the drug. Increase the strength of the solution to 0.5 per cent. Ref.—Lingle; Am. Jour. Physiol., VIII, 75.

3. **Caffeine on the frog's heart.** Expose the heart of a pithed frog and adjust a balanced lever on the ventricle. Irrigate the surface of the heart with physiological saline from a dropping bottle for a few minutes, then change the irrigating fluid to 1 percent caffeine in saline for five minutes, after which return to saline irrigation. Repeat once or twice, then apply caffeine continuously until the maximum effect is obtained. Compare especially the auricle with the ventricle in the later stages of the caffeine effect.

When the heart is perfused through one of the veins then the solution of caffeine should not be stronger than 0.1 to 0.2 percent, and the record

should be taken by the apex suspension method. This method yields the more accurate results.

4. Caffeine on muscular irritability and muscular work. Lay a tight ligature around the thigh of a frog to close off the circulation in one leg. Give a dorsal lymph sac or abdominal injection of **1 c.c. of 0.5 percent caffeine.** Allow twenty minutes for absorption, then pith the frog and ligate off the caffeinized gastrocnemius. Determine the irritability by the minimal stimulus method and, second, determine the amount of work the muscle will do when stimulated directly once in two seconds

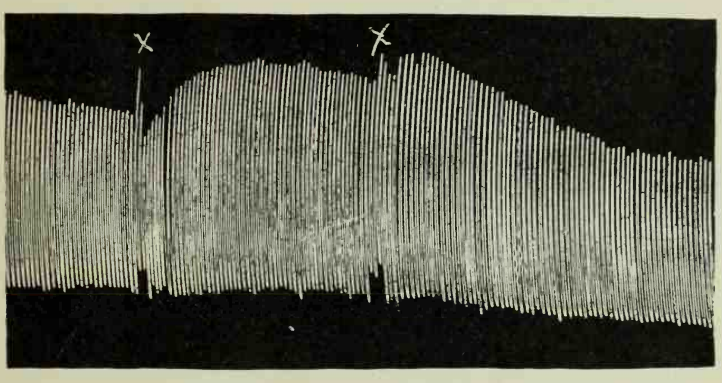

FIG. 12.—Action of caffeine on the ventricular muscle of the terrapin. Between the marks X-X the strip was immersed in 0.5 percent caffeine in saline. Before and after the caffeine the strip contracted in normal saline.

until completely fatigued. Use a constant load of 50 grams in this experiment. Prepare first the normal gastrocnemius as quickly as possible and test its irritability and the amount of work it will do with uniform load and method of stimulation. Repeat the irritability test and the determination of work using the caffeinized muscle. If the records are taken on the same recording paper, one above the other as in experiment 5, page 3, the comparison is very sharp.

5. Caffeine on the voluntary work of human muscle. Measure the amount of work of the flexors of the middle finger by means of a Mosso's ergograph while lifting a 3 to 4 kilo weight, one that exhausts the muscle in about 50 contractions. Repeat in thirty minutes. Consider these as normals. Take two cups of strong coffee or **0.3 gram of caffeine** in sweetened warm water. Remeasure the work of the flexors as directed

above 60 and 90 minutes after taking the caffeine. The amount of the muscular work is usually markedly increased by caffeine. See the next experiment.

6. Caffeine on the reaction time in man. Arrange a tuning fork vibrating one hundred times per second and a set of signal keys for measuring the reaction time to touch. (For details see directions in Stirling's Practical Physiology, page 325.) Determine the normal reaction, then the reaction time at 30, 60, 90 and 120 minutes after a dose of **0.3 gram**

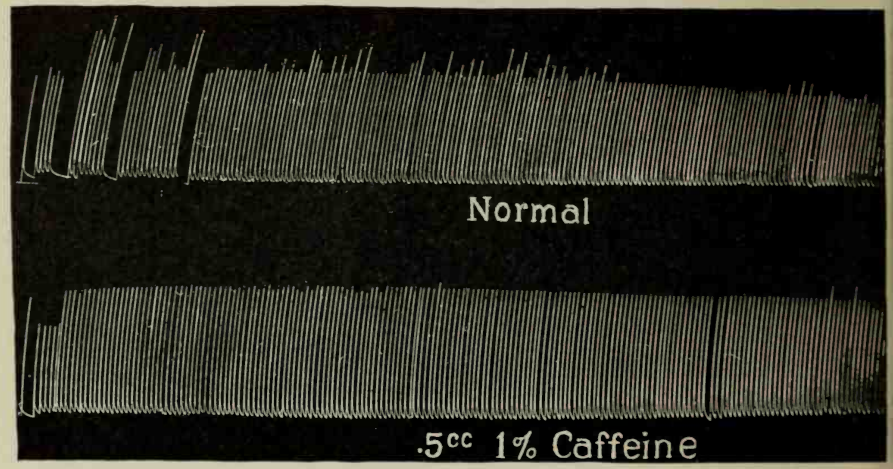

FIG. 13.

FIG. 13.—Caffeine effect on the amount of work of the gastrocnemius of the frog. The upper tracing is the normal, the lower the opposite muscle after the absorption of

caffeine in water. This experiment may be performed together with the preceding.

7. Caffeine on the reflex time in a frog. Prepare a reflex frog by destroying the brain and the medulla. After the shock has passed away determine the reflex reaction time to electrical stimuli applied to the toe of the suspended frog. This time is easily recorded by a paper writing point attached to some part of the foot itself. Give a dose of **1 c.c. of 0.5 percent caffeine** in the dorsal lymph sac. After 30 minutes redetermine the reflex time. Draw conclusions from averages here, as the error of procedure is high.

8. Caffeine on the mammalian heart. Etherize a cat, bleed it, and dilute the blood with Locke's solution as in the alcohol experiment 4. Prepare the apparatus and raise its temperature to 36° C. Isolate the heart and quickly insert it in the cardiograph and start the circulation of the Locke-blood solution. When the rhythm is regular, perfuse with **0.02 to 0.2 percent caffeine** in Locke-blood solution. It is better to begin with a weaker perfusion solution of caffeine and increase the strength through two or three grades, say from **0.1 to 0.5 percent.**

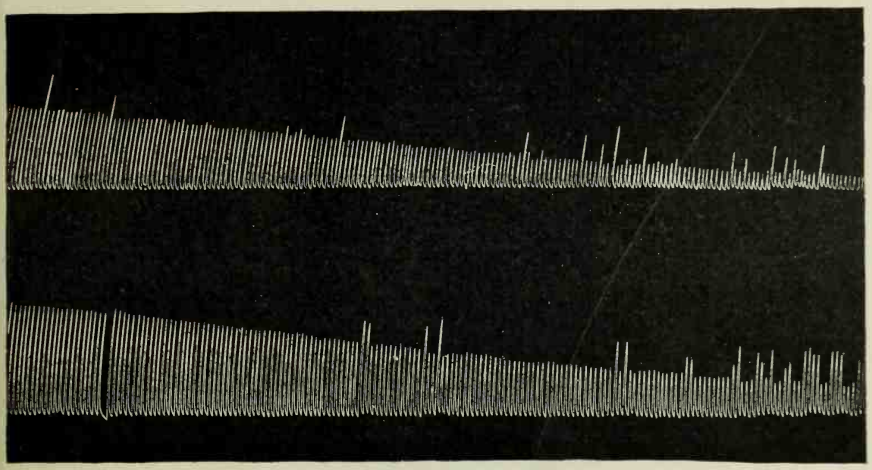

FIG. 13 (Continued).

The stimulus was repeated at regular intervals once in two seconds until exhaustion. 0.5 c.c. 1 percent caffeine.

9. Caffeine on the circulation and respiration of the mammal. Give a dog a hypodermic of 1 c.c. of 2 percent morphine and anesthetize with chloroform. Tracheotomize. Take the arterial blood-pressure from the carotid and the respiratory pressure from the trachea. Insert a canula in the saphenous vein for intravenous injections. Take records on the continuous paper kymograph. After securing a normal record of both the blood-pressure and the respiration give an intraveous injection of **5 to 10 c.c. of 0.5 percent caffeine** in physiological saline. Give slowly from a warmed buret. This experiment should begin with the small doses and the doses be gradually increased. See the next experiment on diuretic action.

10. Caffeine as a diuretic. Prepare a dog as in the preceding experiment. Take the blood-pressure and the respiratory rate. Insert a canula for venous injections. Open the abdomen in the median line, seek out one ureter near its connection with the bladder, ligate and insert a urethral canula, taking care to make an unobstructed connection. After the flow of secretion has been established, a 2 to 3 mm. rubber tube is connected with the canula and the abdominal opening sewed up. Connect the rubber tube with a 1 c.c. pipet graduated in 0.1 c.c. Mount the buret horizontally. Read the rate of secretion by injecting a small bubble of air each five minutes into the rubber tube at its connection with the buret.

Determine the rate of secretion under constant anesthesia both before and after **5 to 10 c.c. of 0.5 percent** caffeine. Take readings every five minutes for a period of about two hours.

STRYCHNINE.

List of Experiments Showing the Effects of Strychnine.

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7. Local action on the spinal cord 26
8. Spasms depend upon cutaneous stimulation 27
9. Absorbed slowly from the stomach or bladder and readily from the intestine or peritoneum 27
10. Stored in the spinal cord 27
11. On the mammalian heart 28
12. On the blood-pressure and respiration rate of mammals. 28

1. Strychnine on the frog. Give a frog a toxic dose of **strychnine nitrate, 0.3 c.c.** (5 minims) **of 0.1 percent.** Give in the dorsal lymph sac. Note the time of the appearance and the successive stages of increased irritability, convulsions, and paralysis. Take fresh frogs and determine the limits of the therapeutic or non-toxic dose, i.e., determine the dose per gram of body weight that will just fail to produce convulsions.

2. Strychnine on the mammal. Demonstration. Give a rabbit a hypodermic injection of **0.4 c.c.** of **0.1 percent strychnine nitrate** per

kilo of body weight. Consult Cushny's Pharmacology for symptoms. Meltzer gives the toxic dose for rabbits as 0.5 mgr. per kilo. Reference, Am. Jour. Physiol., Volume IX, page 1.

3. Strychnine on the ventricular strip. Suspend a strip of terrapin's ventricle in physiological saline and, when it is contracting regularly, subject it to a bath of **0.1 percent strychnine nitrate** in saline for five minutes. Return the strip to physiological saline until the contractions are unquestionably of the saline type. The rhythm and amplitude are both reduced.

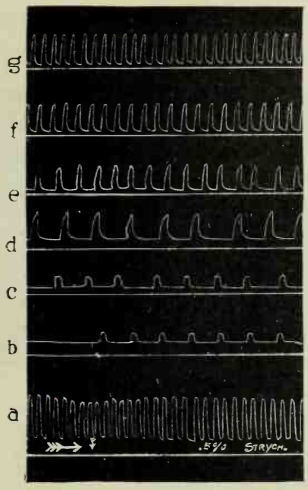

FIG. 14.—Action of strychnine solution on the ventricular muscle of the terrapin. In trace **a** the strip was contracting in physiological saline. At the vertical arrow it was transferred to 0.5 percent strychnine. The contractions are gradually slowed and weakened. Tracings **b** to **g** are successive records around a six-inch drum during the recovery which is very prolonged in this experiment.

4. Strychnine on the heart and cardiac nerves. Pith a frog, expose the heart, take a direct tracing of the movements of the ventricle. Test the vagus activity by stimulating the vago-sympathetic trunk with a current that produces complete inhibition. Next irrigate the surface of the heart from a dropping bottle with **0.1 percent strychnine nitrate** in physiological saline. While continuing the irrigation stimulate the vagus trunk at intervals of 5 to 10 minutes. Look for a progressive effect on the beat, the rate, and on the cardiac nervous mechanism as demonstrated by the results of the stimulation.

5. Strychnine on the irritability and work of voluntary muscle. Ligate the thigh of one leg of a frog to occlude its circulation, give a dose of **0.2 c.c. of 0.1 percent strychnine nitrate.** In twenty minutes ligate off the other leg, pith the frog (pith immediately if tetanic contractions appear earlier), and determine the irritability of the normal and of the drugged gastrocnemii by the minimal stimulus method.

Follow the above test by measurement of the work the muscles will do, testing first the normal, and second, the strychnine muscle. Use the method described for experiment 5 under alcohol. The irritability and the work of voluntary muscle are both greatly increased in the therapeutic stage, in sharp contrast to the effect of strychnine on heart muscle.

FIG. 15—Showing the action of strychnine on the muscular work and on amplitude of the single contractions in the frog's gastrocnemius. The lower series is of the normal muscle, the upper of the strychninized muscle. The dose was 0.25 c.c. of 0.1 percent strychnine hydrochloride injected into the dorsal lymph sac, after ligation of one leg at the thigh. Absorption was allowed until the first spasms when the frog was pithed. Weight of frog 40 grams. Load 200 grams. One stimulus in three seconds.

6. Strychnine on reflex irritability and reaction time. Prepare a reflex frog, i. e., destroy only the brain including the medulla. After the shock has passed away, one hour or more, determine the reflex reaction time to electrical stimulation of the toe by the method given on page 71. Now give **0.5 c.c.** (8 minims) **of 0.02 percent strychnine nitrate.** After each ten minutes take the reaction time to electrical stimulation until spasms appear, which ought to be under 60 minutes.

7. Local action of strychnine on the spinal cord. Cut the cord of a frog at the base of the medulla and destroy the brain. Free the cut end of the cord from the surrounding tissue, and carefully paint it with **1 percent strychnine nitrate** solution. Muscular spasms will be produced or will follow stimulation of the toes of the fore leg. Or stimulate the toe of the

hind leg—reflexes of an orderly nature occur, where no general tetanic convulsions have been induced by the dose. Pith the cord, all spasms cease.

8. Strychnine spasms depend also upon cutaneous stimulation. Strychninize a frog and when the spasms are strong and continuous paint the skin with a **2 percent cocaine** solution. The cocaine paralyzes the cutaneous sensory apparatus whereupon the convulsions cease. Dip the frog in water to remove the excess of cocaine, its local effect will disappear in about 10 minutes and the strychnine convulsions will reappear.

9. Strychnine is absorbed very slowly from the stomach or bladder, but very readily from the intestine and body cavity. Anesthetize a half-grown fasting cat. Ligate both the cardiac and the pyloric orifices

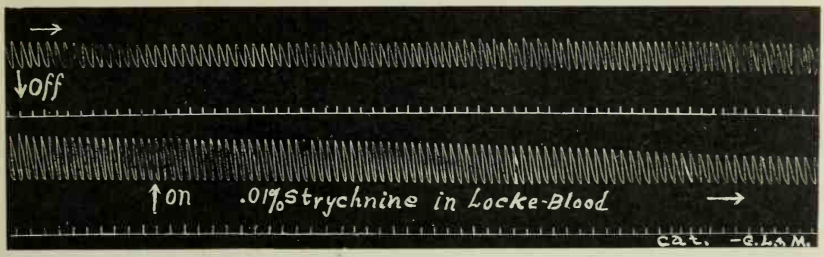

FIG. 16.—Strichnine on the isolated mammalian heart. The heart was perfused with Locke's solution containing 10 percent of the animal's own blood. Between the points marked *on* and *off*, a total of 50 seconds, the heart was profused with 0.01 percent strychnine hydrochloride. The stock solution of the drug had been carefully neutralized to eliminate any trace of acid effects. Temperature 34° C. Time in seconds.

of the stomach. Inject **10 c.c. of 0.1 percent** (10 mgrs.) of **strychnine nitrate into the stomach.** If no spasms occur in 30 minutes then cut the pyloric ligature and run the stomach content into the intestine. Spasms may be looked for in two minutes or less. If enough strychnine is absorbed from the stomach to produce muscular spasms repeat the second half of the experiment on a second animal, injecting the drug directly into a portion of the intestine.

Compare absorption from the urinary bladder and from the abdominal cavity in the same manner.

10. Strychnine is stored in the spinal cord (Lovett, Jour. Physiol., Volume I, p. 99). Inject **10 mgs. strychnine nitrate** into the dorsal lymph sac of a large bull frog. Allow 30 minutes for absorption. Then remove the skin and wash away all traces of strychnine that may re-

main unabsorbed. Take the cord, also an equal amount of other tissue, macerate each in 0.7 percent saline. Inject equal portions of the extracts in the dorsal lymph sacs of two frogs. Allow hours, if necessary, for the symptoms to develop. This method will detect traces of strychnine too small for chemical identification.

11. **Strychnine on the mammalian heart.** Arrange the apparatus for the isolated mammalian heart, bring it to a temperature of 34° to 36° C. Anesthetize a cat or rabbit with ether, bleed and defibrinate the blood and dilute it to ten volumes with Locke's solution. Quickly cut out the heart, insert the canula into the aorta and ligature it, attach the cardiograph, and start the perfusion of Locke-blood solution. Perfuse with **0.005 percent strychnine hydrochloride** for 30 secods. The dose may be increased to 0.01 or even 0.02 percent but recovery from the latter is very slow and gradual.

12. **Strychnine on the blood-pressure and on the respiration rate of mammals.** Anesthetize a 10-kilo dog. Take a continuous record of the blood-pressure from the carotid and of the respiration rate from the trachea. Give an injection of **1 c.c. of 0.1 percent** solution of **strychnine nitrate** from a hypodermic into the saphenous vein. One should give close attention to the symptoms of this mild dose which will produce little more than the therapeutic effects. Repeat this injection until the cumulative dose produces convulsions of a mild character. Note that the convulsions may be suppressed here by giving more chloroform. Give especial attention to the blood-pressure conditions during the tetanic spasm, so as to eliminate the strictly passive mechanical factors.

COCAINE.

List of Experiments Showing the Action of Cocaine.

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5. On muscle work. 30
6. On the circulatory and respiratory systems of the mammal. 31

1. **Cocaine on the frog.** Give a frog a dorsal lymph sac injection **of 0.4 c.c. of 0.5 percent** solution of **cocaine hydrochlorate** in physiological saline. Observe the symptoms in the usual way. Also examine the white corpuscles of the blood for motility as compared with the unpoisoned frog.

2. **Cocaine on local sensory surfaces.** Paint one-half the surface of your own tongue with a brush wet in **2 percent solution of cocaine**

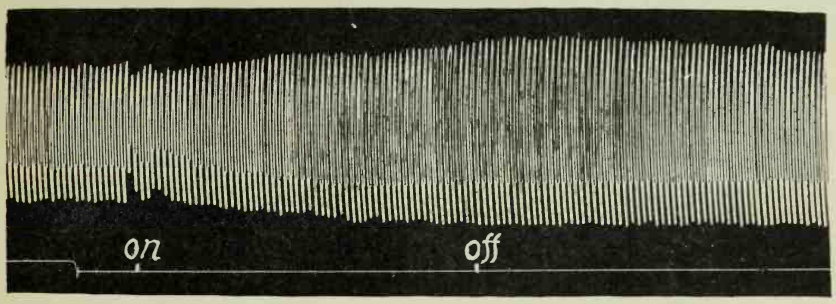

FIG. 17.—Cocaine effects on the frog's heart when perfused in 0.001 percent Ringer's solution. Time in seconds.

hydrochloride. Use care not to swallow any of the solution. In 8 to 10 minutes compare the sensitiveness of the two halves of the tongue to electrical currents by the minimal stimulus method. Test for taste sensations of sweet, of salt. Note also the personal sensations of any character resulting from the experiment.

Give **one drop of 1 percent cocaine** in the right eye; repeat in three minutes. There is a loss of sensitiveness and the eyeball may be

touched without pain. Compare the pupils as to size, as to reaction to light. Determine the acuteness of vision of each eye separately at the reading distance. Cocaine is an analgesic, but not a perfect mydriatic.

3. **Cocaine on the heart muscle.** Prepare a strip of terrapin's ventricle and when it is beating in physiological saline in good rhythm submit it for from three to five minutes to a bath of **0.01 percent cocaine** in saline. Record the contractions on a slow drum.

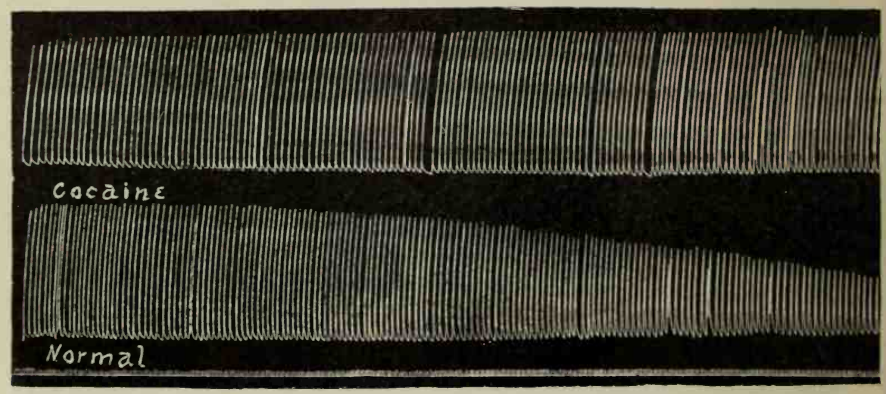

FIG. 18.

FIG. 18.—Effect of cocaine on the muscular work of the frog's gastrocnemius.

4. **Cocaine on the frog's heart and its nervous mechanism.** Pith a frog, expose the heart. Take a continuous record of its contractions by the usual method. Irrigate its surface with physiological saline. Test the irritability of the vagus trunk using a medium strength stimulus. Now irrigate the heart for one minute with **0.2 percent cocaine** in saline followed by saline. When the cocaine contractions have somewhat recovered, retest the inhibitory power of the vagus. Repeat the test,using two or three drops of **1 percent** solution, and do not wash it off afterward.

Perfuse the heart with **0.002 percent** solution of cocaine in Ringer's weaker solution. This method gives more constant results than does the irrigation and it is to be preferred.

5. **Cocaine on muscle work.** Ligate one leg of a frog at the thigh, or use the method described under alcohol. Give a dose of **0.4 c.c.** (7 minims) **of 0.5 percent cocaine** in the dorsal lymph sac. Allow 20 minutes for absorption then ligate the cocainized leg. Load with a 50-grain weight.

Take records of the contractions of the normal muscle on a drum with a speed of 1 mm. per second. Stimulate with single break induction shocks once in two seconds until exhausted. Prepare the cocainized gastrocnemius, mount and stimulate with the same rate and load. If the two records are parallel on the same paper, see figure 18, it will demonstrate the comparative difference in work done. Calculate the amount of work per gram of muscle in each of the two preparations.

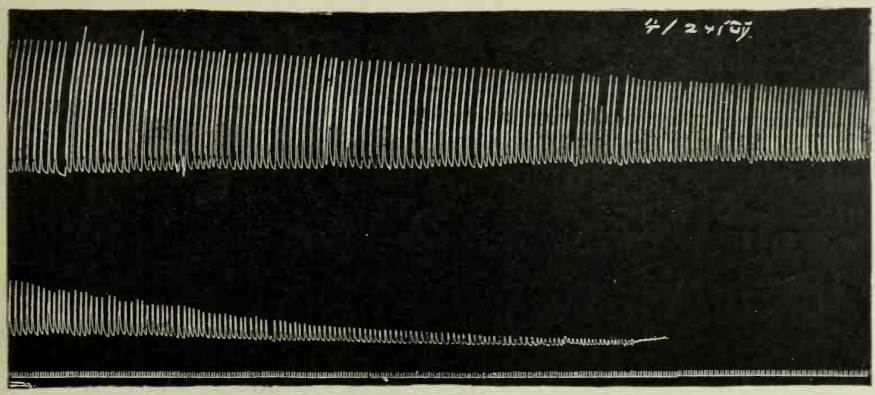

FIG. 18 (Continued).

The endurance of the cocainized muscle is greatly increased.

6. Cocaine on the circulatory and respiratory systems of the mammal. Give morphine and chloroform to a dog. Insert a tracheal canula. Take the blood-pressure and respiratory records on the continuous paper kymograph. Insert a canula and connect a buret with the saphenous vein. Inject **2 c.c. of 1 percent cocaine** very slowly while watching the blood-pressure as an indicator.

QUININE.

Experiments on the Effect of Quinine. PAGE.

1. On the frog. 32
2. On the frog's heart. 32
3. On the striated muscle 32

1. Quinine on the frog. Inject **1 c.c. of 0.1 percent** solution of **hydrochlorate of quinine** into the dorsal lymph sac. In addition to the usual observations, examine the blood of this frog as regards the motility of the white corpuscles. Compare with the blood of a normal frog.

2. Quinine on the frog's heart. Pith a frog, expose the heart, and take a record of its contractions with physiological saline while perfusing it from a perfusion bottle. Change the Ringer's fluid to **0.05 percent quinine hydrochlorate** in Ringer for about one minute. Repeat after

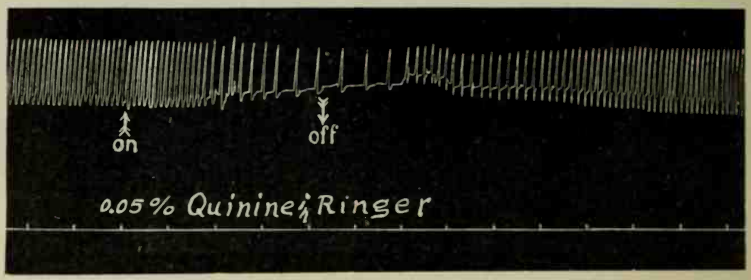

FIG. 19.—Action of quinine on the frog's heart. The perfusion method was used. Normal rate with Ringer's solution. Strength of quinine 0.05 per cent. Time in half minutes.

recovery, using **1 percent quinine.** Continue this perfusion until no further contractions are secured. Examine the condition of the ventricle at the close of the experiment.

Vary this experiment by pithing the frog, taking care to lose little blood and making a record from the ventricle. Now give a lymph sac injection of **1 c.c. of 1 percent quinine** and take a continuous record through 20 to 30 minutes.

3. Quinine on the striated muscle. Ligate one leg of a frog at the thigh, inject **1 c.c. of 0.1 percent** quinine into the dorsal lymph sac. In just 20 minutes ligate off the other leg and pith the frog. Determine the irritability, first of the normal then of the drugged muscle. Determine the work each gastrocnemius will do.

ATROPINE.

Experiments Showing the Action of Atropine.

PAGE.
1. On the frog 33
2. On the heart muscle 33
3. On the frog's heart and on the cardiac nervous apparatus. 34
4. On the secretory nerves of a mammal 34
5. On the isolated heart of the cat. 35
6. On the circulatory and respiratory systems of the mammal. 35
7. On the eye 36
8. On man in the therapeutic dose 36
9. As secreted by the kidney 36
10. Scopolamine on the frog 36

1. **Atropine on the frog.** Give a frog an injection of **1 c.c. of 1 percent of atropine sulphate.** Keep it under observation in a moist battery jar until complete recovery. The toxic dose is **1 c.c. of 3 percent.**

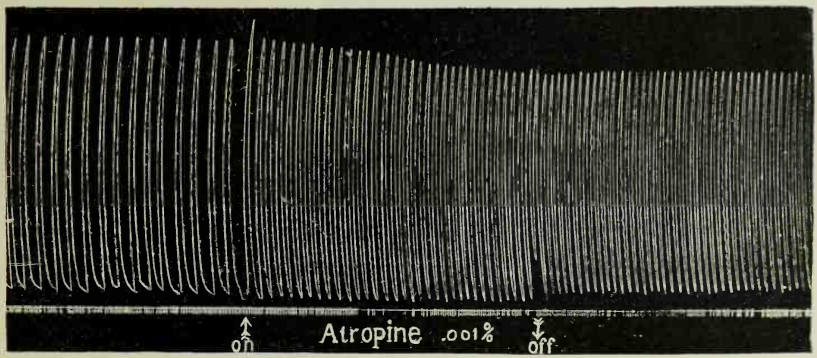

FIG. 20.—Action of atropine on the heart muscle of the terrapin's ventricle. The normal solution is sodium chloride, the strength of atropine 0.001 percent. The decrease in amplitude is usually not so great as in this tracing, or is even absent entirely Time in seconds.

2. **Atropine on the heart muscle.** Mount a ventricular strip from the terrapin in 0.7 percent sodium chloride, and when it is contracting with a uniform amplitude and regular rhythm change to **0.001 percent atropine** in physiological saline for five minutes, see figure 17. Recover the characteristic rhythm in saline and repeat using **0.002 percent atropine** in saline.

3. Atropine on the frog's heart and cardiac nervous apparatus. Pith a frog, expose the heart and take a tracing. Determine an effective strength of stimulus for the vagus. Now irrigate the heart for one minute from a dropping bottle containing **0.1 percent atropine** in saline. Stimulate the vagus immediately and once every five minutes or less. Atropine eliminates the vagus control of the heart by poisoning the peripheral endings. The atropine effect is antagonized by physostigmine, page 44, experiment 3, and by muscarine.

4. Atropine on the secretory nerves of a mammal. Anesthetize a 10 kilo dog with morphine and chloroform. Expose and tie a canula in

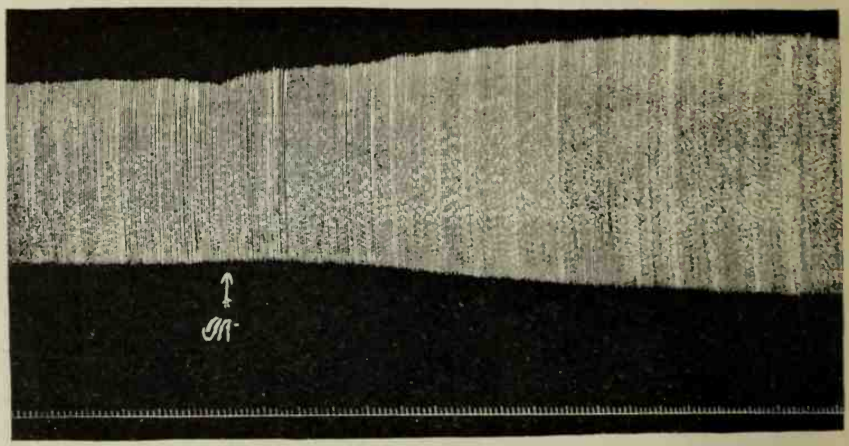

FIG. 21.

FIG. 21.—Action of atropine on the isolated heart of the cat. Locke-blood solution the arrows was .001 percent. Temperature 39° C. Pressure 85 cm. of water. The

the submaxillary duct. Expose and stimulate the chorda tympani nerve in the hilus of the gland, noting the rate of secretion by the drops of saliva per minute from the canula. Give a hypodermic injection of **1.5 c.c. of 1 percent atropine.** Stimulate the chorda tympani nerve again. No secretion is obtained even though the nerve is stimulated down close to the hilus of the gland.

This demonstration may be made in part as follows: Produce a rapid flow of saliva in the dog by a hypodermic of **1 c.c. of 0.2 percent pilocarpine.** Observe the flow by turning out the dog's upper lip. Follow with a hypodermic dose of **1 c.c. of 1 percent atropine.** The secretion stops.

5. Atropine on the isolated heart of the cat. Anesthetize a cat with ether. Bleed it and prepare the Locke-blood perfusion fluid. Isolate and suspend the heart in the perfusion apparatus. Obtain a normal record then perfuse with **.001 percent atropine** in Locke-blood solution.

6. Atropine on the circulatory and respiratory systems in the mammal. Anesthetize a 10-kilo dog as in experiment 4 preceding. Place an arterial canula in the carotid and insert a tracheal canula. Take a continuous record of the blood-pressure and of the respiration. Stimulate the peripheral end of the sectioned vagus with a stimulus that produces complete inhibition of the heart. Stimulate also the central end of the vagus. Now

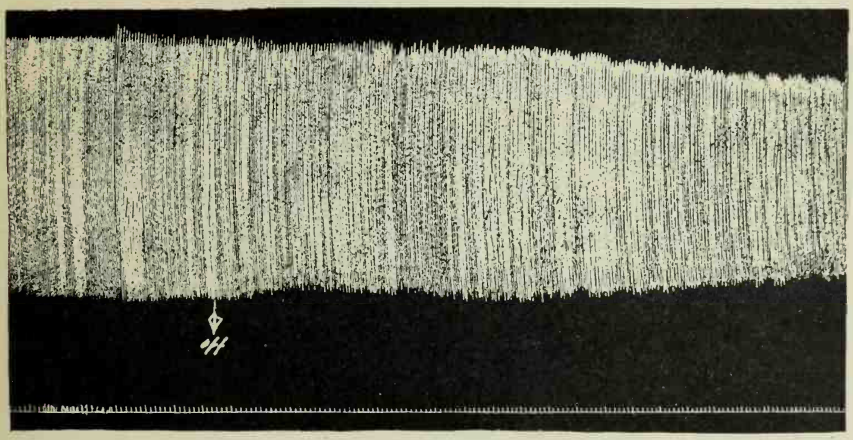

FIG. 21 (Continued).

was used for the normal perfusion fluid. The strength of atropine perfused between time in seconds.

give an intravenous injection of **1 c.c. of 0.1 percent atropine.** Note the exact time of the injection on the record by a signal pen. When the equilibrium is again established, re-stimulate the ends of the sectioned vagus with the same strengths of stimulus used before atropine was given. Note that the heart rate is no longer slowed on stimulation of the peripheral vagus, but that the pupil still actively dilates after this dose when the central end of the vagus trunk is stimulated. Atropine also destroys the vagus control over the smooth muscle of the alimentary tract thus decreasing its motility.

Physostigmine is an antagonist to atropine. Try **1 c.c. of 0.1 percent**

intravenous. Use artificial respiration if necessary. Give a second injection of atropine later. See figure 23 for a reverse antagonism.

7. Atropine on the eye. Drop **1 or 2 drops of 1 percent atropine** in the right eye of a dog or cat. The pupil will be widely dilated in a few minutes. Keep the animal under observation until the effect entirely disappears, often only after several days. Atropine destroys the power of accommodation and it is used for this clinical purpose in eye practice. Students should not use atropine on their own eyes, but a mild dose of **homatropine, 2 or 3 drops of 1 percent,** the effect of which passes off in 24 to 36 hours, may be tested in one's own eye. In such experiments test the accommodation, light reflex, and size of the pupil.

8. Atropine on man in therapeutic dose. Test on yourself the action of a dose of **1/120 to 1/60 grain of atropine** by way of the mouth. Note the effects on the heart rate, pulse character, respiration, size of pupil, light reflex and sensations.

9. Atropine is secreted by the kidney.—This may be demonstrated on the rabbit which is very tolerant of the drug. Give a rabbit urethane. Collect the urine from a bladder canula. Give a large hypodermic injection, **2 c.c. of 2 percent atropine,** and test the rabbit's urine on the eye of a cat or dog. The atropine may be extracted (Binz). Concentrate a large amount of urine, add ammonia, shake up with chloroform, evaporate, dissolve the residue and test on the eye of a cat or a dog.

10. Scopolamine on the frog. Give a dose of **1 c.c. of 1 percent scopolamine** in the dorsal lymph sac of a frog. Compare with the effects of an equal dose of atropine in experiment 1 above.

NICOTINE.

Experiments Illustrating the Action of the Nicotine.

PAGE.
1. On the frog. 37
2. On the ventricular muscle. 37
3. On the frog's heart and its nervous apparatus. 37
4. On the nerve fiber and on nerve ganglia. 37
5. On the mammalian heart. 38
6. On the circulatory system and on the respiratory nervous mechanism 38
7. On muscle irritability 39

1. Nicotine on the frog. Give an injection of a **0.5 c.c. of 0.2 percent nicotine** into the dorsal lymph sac of a frog.

2. Nicotine on the ventricular muscle. Prepare a terrapin's heart strip and when it is contracting rhythmically in 0.7 percent physiological saline, immerse the strip in a **0.05 percent solution of nicotine** in saline for two minutes, then return to the saline bath. Repeat. If the solution is too strong the strip will exhibit a strong tonus with incomplete relaxations. The amplitude and the rate are markedly increased.

3. Nicotine on the frog's heart and its nervous mechanism. Pith a frog, expose the heart and take tracings on a drum with a speed of 2 mm. per second. Stimulate the vago-sympathetic with an interrupted current that just causes complete inhibitions. Now irrigate the heart from a dropping bottle with **0.1 percent nicotine** in 0.7 percent saline, and stimulate the vagus at intervals of two minutes. If the nerve stimulation ceases to be effective, then apply the electrodes directly to the sinus.

To demonstrate the stronger effects on heart muscle prepare a second frog. Take a tracing of the heart. Apply a few drops of **1 percent solution of nicotine.**

4. Nicotine on the nerve ganglia and on the nerve fiber. Anesthetize a rabbit (or cat or dog), dissect out the cervical sympathetic and the superior cervical ganglion. Stimulations of the nerve or of the ganglion lead to vasoconstriction in the ear and dilation of the pupil. Paint the nerve below the ganglion with **1 percent nicotine.** Stimulation at a point still lower down shows that the nerve impulses still pass undisturbed. Now paint the ganglion itself. Stimulate the nerve below the ganglion, also

the ganglion directly. What Conclusions? See also the next experiment. (Ref.: Langley and Dickinson, Journal of Physiol., Volume II, page 265.)

5. Nicotine on the mammalian heart. Prepare the mammalian heart perfusion and recording apparatus and bring it to a temperature of 36° C. Etherize a cat, bleed, defibrinate the blood, and dilute to one-in-ten of Locke's solution. Quickly take out the heart, suspend it in the apparatus and start the perfusion. When the heart is beating well, perfuse it with **0.001 percent of nicotine** in Locke-blood solution. There is a constant

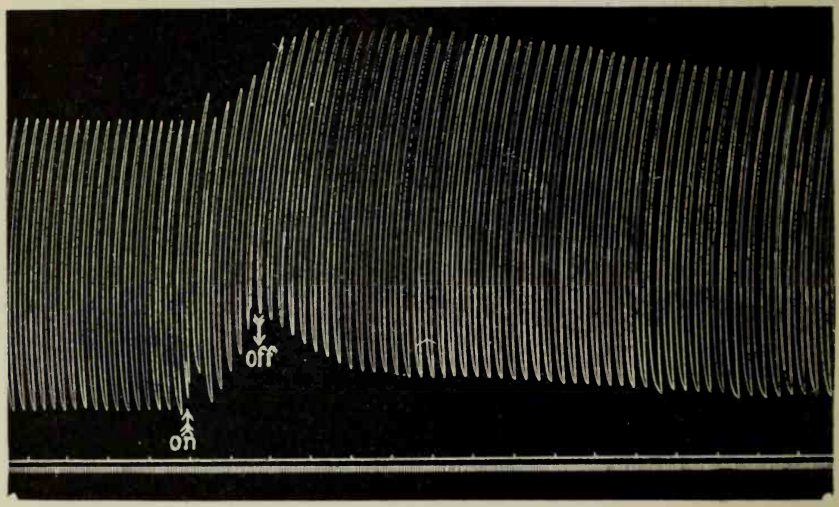

FIG. 22.—Action of nicotine on the ventricular muscle from the terrapin. The strip was contracting in physiological saline when transferred to 0.2 percent nicotine in saline at the point marked. The solution was too strong and was removed after about forty seconds. Time in seconds and half-minutes.

sharp increase in amplitude with a more slowly developed increase in rate. The after amplitude may remain greater than the preceding normal.

6. Nicotine on the circulatory and respiratory nervous mechanism. Anesthetize a dog or cat (the animal used in experiment 4 above may be used for this experiment also). Take a blood-pressure from the carotid and a respiration tracing from the trachea. (1) Determine an effective stimulus for the heart and respiration. (2) Now inject **5 c.c. of 0.1 percent solution of nicotine** into the saphenous or jugular vein. Repeat

the dose if necessary, until distinct effects are produced on the heart rate and blood-pressure. (3) Stimulate the vagus at first with the strength of stimulus used before the injection, then with successively stronger stimuli. An instructive picture is obtained by dissecting down to and stimulating the cardiac branches from the annulus of Vieussens, which may be done in the dog on the left side without opening the thorax.

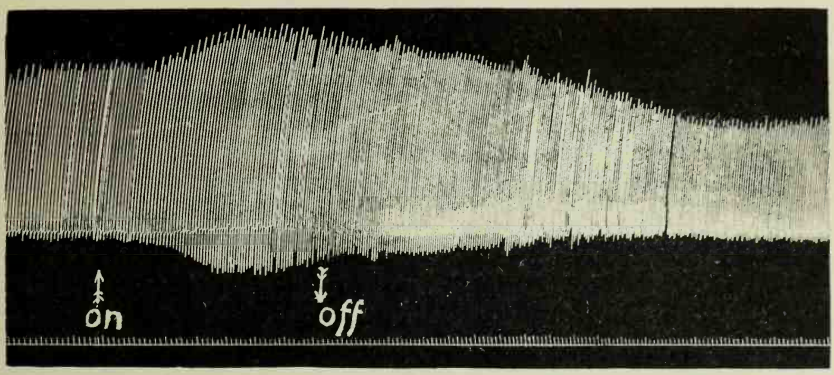

FIG. 23.—Action of nicotine on the isolated cat's heart. The heart was contracting in Locke-blood solution when it was perfused with 0.001 percent nicotine between the points marked by the arrows. The increase in amplitude is more marked when the heart is beating weaker at the time of perfusion. Time in seconds.

7. Nicotine on muscle irritability. Lay a ligature around the thigh of one leg of a frog and then give **1 c.c. of 0.1 percent nicotine** in the dorsal lymph sac. After 20 minutes test the irritability of the normal and of the nicotinized gastrocnemius muscles by the minimal and maximal stimulus method.

CURARE.

Experiments on the Effect of Curare. PAGE.

1. On the frog. 40
2. On the motor nerve endings. 40
3. On the heart muscle and on the cardiac nervous mechanism. 40
4. Poisons the motor endings before the other portions of the reflex arc. 41
5. On the mammal. 41

1. Curare on the frog. Give a frog a hypodermic of **0.3 c.c.** (5 minims) **of 0.2 percent curare.** The motor apparatus is paralyzed, but the circulation continues and the frog will recover in from one to three days, respiration being maintained through the moist skin if the animal is kept in a covered jar.

2. Curare on the motor nerve endings, Bernard's experiment. The specific action of curare was demonstrated by Claude Bernard to be on the motor nerve end plates. Ligate one leg of a frog to shut off the circulation, give a hypodermic of **0.3 c.c. of 0.2 percent curare.** When general paralysis is secured, perform the following tests, interpreting the results through the effect on the gastrocnemius muscles:

a. Stimulate the sciatic nerve on the unligated leg.

b. Stimulate the gastrocnemius of the unligated side.

c. Stimulate the sciatic nerve on the ligated side above the ligature.

d. Below the ligature.

e. The corresponding muscle. Conclusions.

3. Curare on the heart muscle and on the cardiac nervous apparatus. While minimal doses of curare suffice to poison the motor end plates, it takes relatively large doses to paralyze the cardiac nervous apparatus. The paralysis apparently affects the ganglionic nerve endings first and then the cardiac motor endings and muscle. Pith a frog, expose the heart, prepare the vagus trunk for stimulation and adjust a heart lever for record. Allow physiological saline from an irrigating bottle to run over the heart. Take a normal record and then stimulate the vagus nerve. Now irrigate slowly with **0.2 percent curare** in saline and stimulate the nerve 10 seconds at a time at intervals of 10 minutes for several tests.

4. Curare poisons the motor endings before the other portions of the reflex arc. Tie a ligature on a frog's leg at the thigh, inject **0.3 c.c. of 0.2 percent curare.** Just as voluntary activity ceases stimulate the skin of the poisoned leg. The unpoisoned gastrocnemius will contract. Rapidly expose and stimulate the poisoned sciatic. The poisoned gastrocnemius will not contract, while the unpoisoned one will, owing to reflex stimulation through the cord.

5. Curare on the mammal. Morphinize and chloroform a dog. Take blood-pressure. Introduce a tracheal canula and take the respiratory record by the intra-tracheal method. Arrange the apparatus for artificial respiration when needed. Inject into a vein **5 c.c. of 1 percent curare.** All movements of voluntary muscles will quickly cease including respiratory movements. The heart rate and the blood-pressure remain good, and if artificial respiration is applied the circulation can be maintained for several hours, or until the drug is eliminated and recovery occurs.

PILOCARPINE.

Experiments Showing the Action of Pilocarpine.

PAGE.
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2. On the mammal 42
3. On the ventricular muscle 43
4. On the frog's heart 43
5. On the circulatory and respiratory systems of the mammal . 43

1. Pilocarpine on the frog. Give a frog a hypodermic injection of **0.6 c.c. of 10 percent solution of pilocarpine nitrate.** Keep the frog in a moist battery jar until normal again. The toxic dose is **1 c.c. of 10 percent solution of pilocarpine.**

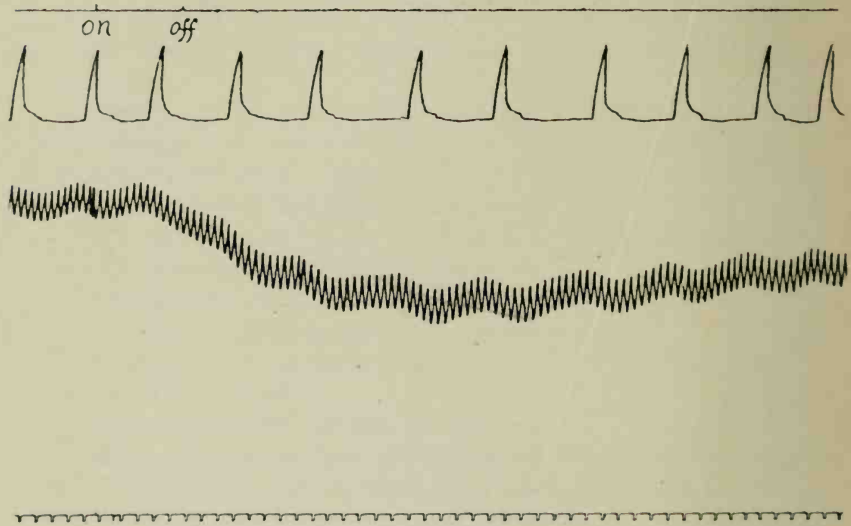

FIG. 24.—Blood-pressure and respiratory effects of an intravenous injection of 1 c.c. of 1 percent pilocarpine in the dog. Injection between the points "on" and "off." Time in seconds. Pressure in mercury. Reduced to four-fifths the original size.

2. Pilocarpine on the mammal. Give a dog a hypodermic injection of **0.3 c.c.** (5 minims) **1 percent pilocarpine.** This dose produces a marked secretion by glandular structures. Examine the flow of saliva by turning back the upper lip, drying it and noting the accumulation of drops

at the mouth of the salivary duct. An injection of **1 c.c. of 0.1 percent atropine** in a vein antagonizes **pilocarpine** and stops the secretion.

Give additional drops of **1 percent pilocarpine** in the eye. Drops of **1 percent atropine** on the eye will overcome the action.

3. Pilocarpine on the ventricular muscle. Mount a strip of the terrapin's ventricle in physiological saline. When the contractions are regular transfer to a **0.1 percent pilocarpine** solution in saline. Allow it to act only 1 minute then renew the physiological saline bath. As a final test give the strip a continuous bath of **0.1 percent pilocarpine** and note the successive effects.

4. Pilocarpine on the frog's heart. Pith a frog, expose the heart and take tracings of the ventricle. Test the activity of the vagus with a strong interrupted current. Now irrigate the surface of the heart with drops of **1 percent pilocarpine** for two minutes. Stimulate the vagus trunk one minute after pilocarpine and at successive intervals of five minutes. If the drug is strongly active the heart will beat slower. In the early stages the stimulation will result in acceleration, but in no inhibition as in the normal. Applying the electrodes directly to the sinus gives no inhibition showing that the pilocarpine has acted on the vagus endings and not on the ganglionic connections. Drops of **1 percent atropine sulphate** will restore the heart beat after pilocarpine, these two drugs being antagonists.

5. Pilocarpine on the circulatory and respiratory system of the mammal. Anesthetize a dog with morphine and chloroform. Introduce a tracheal canula and be prepared to use artificial respiration if necessary. Take blood-pressure and respiration records on a continuous paper kymograph. Test the activity of the vagus. Give an intravenous injection of **1 c.c. of 1 percent pilocarpine nitrate.** Retest the activity of the vagus after the pilocarpine. Atropine antagonizes the pilocarpine effect. Examine the pupils from time to time. Also note the increased rate of salivary secretion.

PHYSOSTIGMINE.

Experiments on the Action of Physostigmine. PAGE.

1. On the frog 44
2. On cardiac muscle 44
3. On the frog's heart and its nervous apparatus 44
4. On the heart of the cat 44
5. On the respiratory medullary center and on the circulatory system of the mammal 44
6. On the eye 47

1. Physostigmine on the frog. Give a frog a dorsal lymph sac injection of **1 c.c.** (17 minims) **1 percent physostigmine.** The effects produced are diminished irritability, loss of muscular tone, paralysis of the respiratory center, loss of reflexes, and death, or, at best, a very slow and prolonged recovery.

2. Physostigmine on cardiac muscle. After a ventricular strip from the terrapin has begun beating regularly in physiological saline, transfer it to **0.1 percent physostigmine** in saline for two to three minutes. Physiological saline recovers the normal contractions after several minutes. Compare the results with those from pilocarpine and muscarine. A bath of 0.002 percent atropine antagonies the physostigmine effects.

3. Physostigmine on the frog's heart and its nervous apparatus. Pith a frog and take a record of the heart beat. Determine the minimal effective stimulus of the vagus trunk for inhibition of the heart rate. Now irrigate the surface of the heart with drops of **0.1 percent physostigmine** from an irrigating bottle for two minutes. Redetermine the minimal stimulus for the vagus trunk beginning with a very weak induction current. If the contractions are at a slow rate or have ceased, irrigate the surface of the heart with **1 percent atropine.** Atropine antagonizes physostigmine. Compare with pilocarpine. If the perfusion method is used then **0.01 percent physostigmine** is the proper solution strength for the frog's heart.

4. Physostigmine on the isolated heart of the cat. Prepare an isolated cat's heart by the method used in the nicotine experiment 5. When it gives regular contractions, perfuse it with **0.01 percent physostigmine** in Locke-blood solution.

5. Physostigmine on the respiratory medullary center and on the circulatory system of the mammal. Anesthetize a dog, insert a trach-

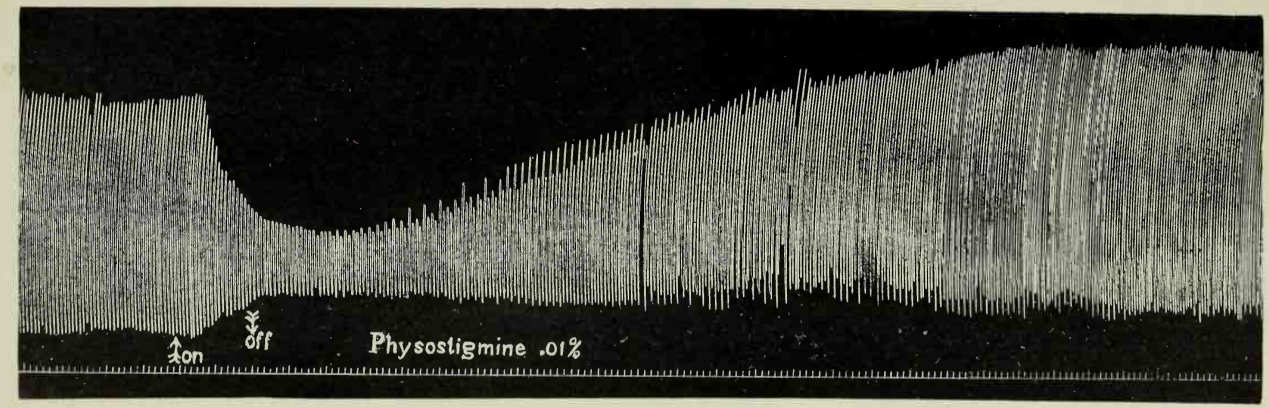

FIG. 25.—Perfusion of 0.01 percent physostigmine through the coronary vessels of the isolated heart of the cat. Time in seconds.

eal canula and be prepared for artificial respiration. Take a continuous record of the blood-pressure and of the respiratory movements. Insert a canula in the saphenous vein and connect it with a buret containing the drug. Give an intravenous injection of **0.1 percent physostigmine** slowly until the first effects are noticed on blood-pressure. Note the amount and mark the time of the dose on the record by a signal pen. Usually there is a pro-

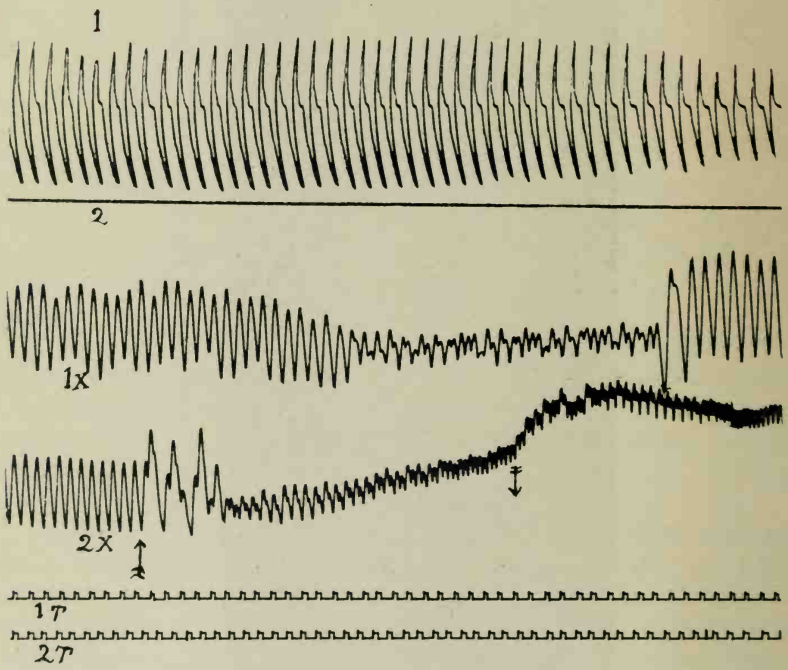

FIG. 26.

FIG. 26.—Action of physostigmine and its antagonism by atropine in the mammal. showing the effects of an injection of 8 mgr. of physostigmine just before the part of of respiration and the circulation effects upon the injection of 2 mgr. of atropine be- At **a**, **b** and **c** are shown portions of the trace at 30-second intervals. Time in seconds.

gressive paralysis of the respiratory center accompanied by a great slowing of the heart and a fall of blood-pressure by one-half or more. The heart continues to beat long after respiration ceases. Use artificial respiration until the blood-pressure improves and the anesthesia becomes light. This will not restore automatic respiratory movements as it does after heavy anesthesia. If at this time a venous injection of **0.5 c.c. of 1 percent**

atropine be given from a hypodermic syringe the respiratory movements will be quickly established and the slow heart and low pressure will give way to the rapid heart and strong pressure following primary injection of atropine, experiment 6, page 35. The vagus inhibitory apparatus is effective under physostigmine, but not after atropine. Repeat the experiment. It takes a larger dose of physostigmine to overcome the atropine and pro-

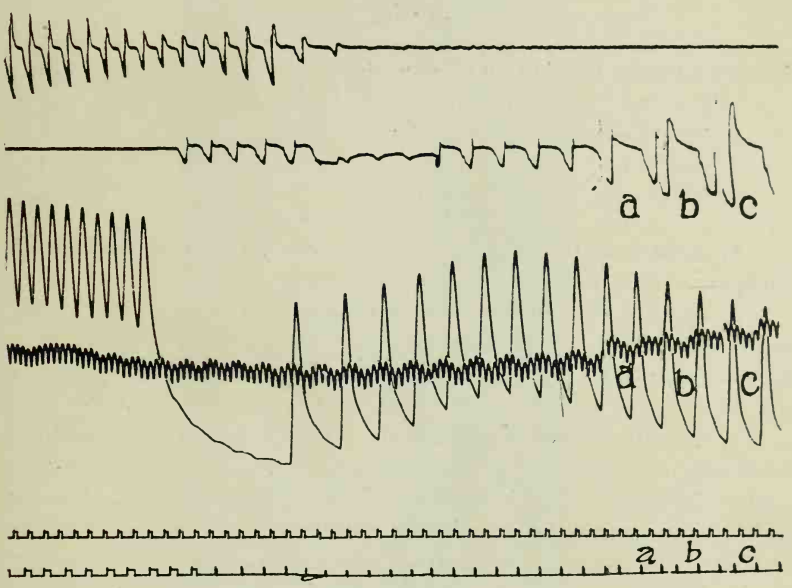

FIG. 26 (Continued).

Parts 1, 1X and 1T give the respiration trace, blood-pressure and time, respectively, the trace shown. The parts of the trace numbered 2, 2X and 2T show the recovery tween the arrows. A few second's gap intervenes between the two parts of the figure.

duce the characteristic effects. Examine the pupil before and after the physostigmine.

6. Physostigmine on the eye. Give **2 drops of 1 percent physostigmine** in one eye of a dog or a rabbit, at intervals of five minutes. It is better to use one of the experimeter's own eyes. Strong contraction of the pupil follows. A decrease in intraocular pressure has also been proven,

and to produce this effect is the chief therapeutic use of the drug. A striking comparison is obtained by dropping **1 percent atropine** in the unused eye of the dog after the physostigmine effect has come on in the other eye. Physostigmine will overcome the atropine dilation of the pupil. The experimenter may show the antagonism between homatropine and physostigmine on his own eyes, but it is recommended that one eye always be reserved.

ACONITE.

Experiments on the Action of Aconite. PAGE.

1. On the frog. 48
2. On the circulatory system of a mammal. 48
3. On the frog's heart. 48
4. On the mammalian heart. 48

1. Aconite on the frog. The dose is **0.5 c.c. of 0.1 percent aconite.** Compare with digitalis.

2. Aconite on the circulatory system of the mammal. Take a continuous tracing of the blood-pressure of a dog. Give **1 c.c. of 0.1 percent aconitine** crystals. Note particularly the progressive effects on the nervous and muscular elements of the circulatory apparatus.

3. Aconite on the frog's heart. Destroy the cerebrum and optic lobes only of a frog, expose the ventricle and take a tracing. Give an injection of **0.5 c.c. of 0.1 percent aconitine** in the lymph sac. One may expect a progressive stimulation of the accelerator and vagus nervous apparatus followed by paralysis of nerves and muscle.

4. Aconite on the mammalian heart. Prepare an isolated heart as described for the nicotine experiment 5. When the heart is contracting regularly with the Locke-blood perfusion then perfuse for 10 seconds with a **0.0002 percent aconite** solution. A prolonged perfusion or perfusion with a stronger concentration of aconite will quickly set up incoordinate contractions and fibrillation.

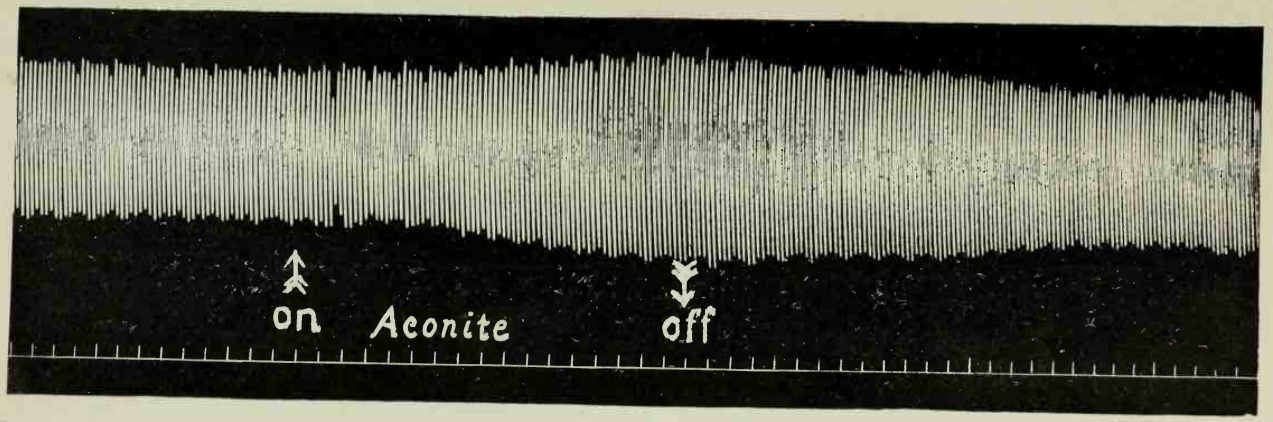

FIG. 27.—Action of 0.0002 percent aconite on the isolated cat heart. Perfusion pressure, 81 cm. of water. Temperature 38° C. Time in seconds.

VERATRINE.

Experiments on the Action of Veratrine. PAGE.

1. On the frog. 50
2. On the mammal. 50
3. Veratrine on heart strip. 50
4. On the frog's heart. 50
5. On the isolated mammalian heart. 50
6. On the form of the simple muscle contraction. 50
7. On the circulatory and respiratory systems of the mammal.. 50

1. **Veratrine on the frog.** The dose for a frog is about **0.5 c.c.** of a **1 percent** solution of the **fluid extract veratrum viride** or **0.3 c.c. of 0.01 percent veratrine.** Compare with the effects of aconite and of barium. See experiment 4.

2. **Veratrine on the mammal.** Give a cat or rabbit **1 c.c. of 0.1 percent veratrine** hypodermically, or **1 c.c. of 1 percent** for a dog. Keep under observation for a considerable time.

3. **Veratrine on the heart strip.** Subject the contracting strip of ventricle to **0.005 to 0.05 percent veratine** in saline.

4. **Veratrine on the frog's heart.** Pith a frog, expose the heart and take a tracing when perfused with **0.005 percent veratrine** in Ringer's solution (0.01 percent destroys coordination).

5. **Aconite on the isolated mammalian heart.** Prepare the apparatus for the isolated heart experiment, isolate a cat's heart and perfuse with **0.0002 percent veratrine** in Locke-blood solution. See Fig. 25.

6. **Veratrine on the simple muscle contraction of the frog.** Ligate one leg of a frog and give a hypodermic of **0.5 c.c. of 0.1 percent veratrine.** After 15 minutes prepare the veratrinized muscle and take simple muscle contractions to show the form of the contraction wave, using a tuning fork to record the drum speed. Compare this curve with that of the undrugged muscle.

The frog of experiment 1 may be used to show the veratrine effect on muscle work. Stimulate once in three seconds in this experiment, since the relaxation may not be complete in an interval of two seconds.

7. **Veratrine on the circulation and respiration of a mammal.** Take a record of the blood-pressure from the carotid of an anesthetized dog. Tracheotomize and take respiratory tracings. Give **1 c.c. of 1 percent veratrine** in the abdominal cavity. When marked cardiac slowing appears cut the vagi and note the effects on the heart.

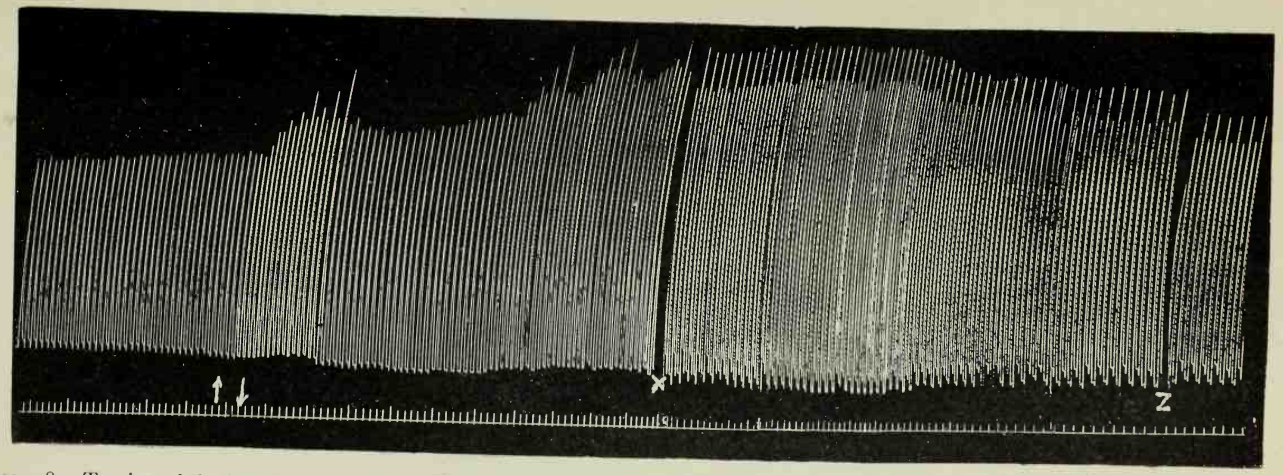

FIG. 28.—Tracing of the isolated cat's heart. Between the arrows a perfusion of 0.0002 percent of veratrine in Locke-blood solution was used for four seconds. Gaps of 30 seconds at **X**, and of 60 seconds at **Z** are shown. Time in seconds.

DIGITALIS.

Experiments Showing the Action of Digitalis.

PAGE.
1. On the frog. 52
2. On the ventricular muscle. 52
3. On the frog's heart. 52
4. On the atropinized frog's heart. 52
5. On the mammalian heart. 52
6. On the circulatory and respiratory systems of the mammal. 53
7. Digitalis as a diuretic 53

1. **Digitalis on the frog.** Give a dose of **0.5 c.c. of 0.2 percent of soluble digitalis.** The digitalis effects develop slowly. Note the heart rate, and particularly the circulation in the web. Keep in a moist battery jar for at least two hours. Examine the heart if death occurs.

2. **Digitalis on the ventricular muscle.** Treat a strip of terrapin's ventricle contracting in saline to a bath of **0.002 percent digitalis** in saline. Follow with pure saline. Repeat with a **0.005 percent digitalis.** Still stronger solutions may be used, but a marked tone will result as shown in Figure 26.

Digitalis solutions may be used made up in the weaker Ringer, but as the rate is slower and the amplitude much greater than that in sodium chloride solutions the picture will be quite different, though the same in kind. Delirium cordis of the strip is produced by the stronger solutions acting for several minutes.

3. **Digitalis on the frog's heart.** Pith a frog and take a record of the contractions of the ventricle when irrigated with physiological saline. Irrigate slowly with drops of **0.2 percent digitalis** for two minutes, then wash off with saline.

A more effective method is to perfuse the heart from a canula in the vena cava. Use a much weaker solution for perfusion, i.e., **0.0005 to 0.001 percent digitalis** in Ringer. These effects should be compared with the effects on cardiac muscle above.

4. **Digitalis on the atropinized frog's heart.** Atropinize the frog's heart to eliminate the cardiac nervous control, then repeat experiment 3 above.

5. **Digitalis on the mammal heart.** Use the method described on page 70. Isolate and perfuse the cat's heart with the normal solution

of Locke-blood, then with **0.0001 percent digitalis** (soluble digitalis) in Locke-blood. Increase the strength to **0.0005** percent. The stronger solution will usually produce a great increase in amplitude followed by fibrillation. The weaker solution, see figure 30, produces a typical mild therapeutic effect on the heart. Compare with the results of experiment 6.

6. Digitalis on the circulatory and respiratory systems of the mammal. Anesthetize a dog and take continuous kymographic records of the blood-pressure and of the respiration. Slowly inject into the saphenous vein **2 c.c. doses of 0.5 percent digitalis at** five-minute intervals

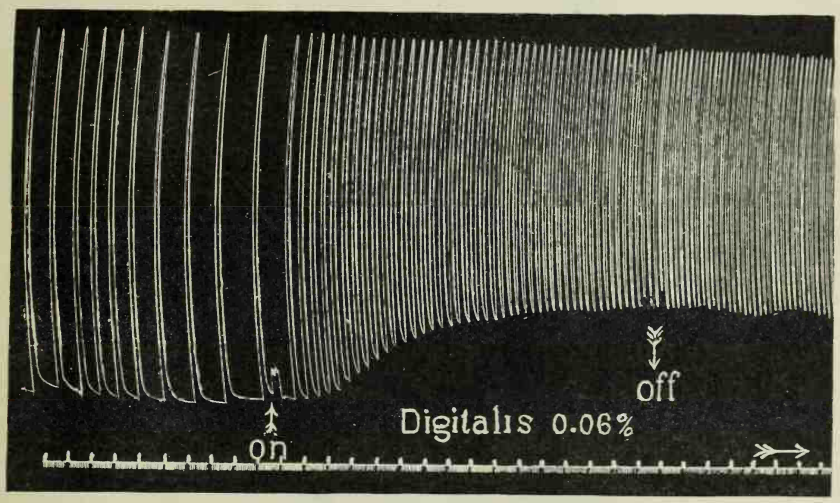

FIG. 29.—Experiment showing the action of digitalis on the rhythm and tone of a strip of terrapin's ventricle. The strip was contracting in physiological saline. Between the words "on" and "off" it was subjected to 0.06 percent of digitalis in saline.

until the three stages of digitalis effect on the heart and blood-pressure are obtained. The anesthetic must be perfectly constant. One may give the maximal dose of **4 c.c. of 1 percent** digitalis at once. In this instance the three stages are passed through rapidly and the animal will usually die in 10 to 20 minutes. Read Cushny's Pharmacology, pp. 430–435.

7. Digitalis as a diuretic. Morphinize and chloroform a dog. Take the blood-pressure. Isolate the ureters near the bladder and insert canulas, using care not to occlude the ureters by twisting or otherwise. Connect the ureters by means of a T-tube with a horizontal 2 c.c. pipet graduated

FIG. 30.—Action of digitalis, 0.0001 percent, on the isolated cat's heart when perfused with Locke-blood mixture. The digitalis perfusion was between the points marked by the arrows. Time in seconds and six-second intervals. Pressure, 90 cm. in water. Temperature, 38° C.

to 1/50 c.c. Close the abdomen with sutures. Insert a venous canula and connect with a transfusion buret. Establish the normal secretion per 10 minutes, cutting off the column of secreted urine by injecting a bubble of air into the mouth of the buret by inserting a hypodermic needle through the rubber connecting tube. Now inject **5 c.c. of 0.1 percent digitalis** or **strophanthin** into a vein and take the secretion in successive 10 minute periods until the flow is constant. Repeat the dose once or twice at long intervals. Mark the secretion intervals on the blood-pressure record.

Compare these results with those observed on other diuretic drugs—caffeine, urea, inorganic salts, etc.

ERGOT.

Experiments on the Action of Ergot. PAGE.

1. On the frog 55
2. On the heart muscle. 55
3. On the arterioles of the frog. 55
4. On the blood-pressure and heart rate of a mammal. . . . 55

1. Ergot on the frog. Give **0.5 c.c.** of the fluid extract.

2. Ergot on the heart muscle. Change a contracting heart strip from saline to a **10 percent** solution of Squibbs' fluid extract of ergot in saline solution. Allow it to act for five minutes. Take a continuous record.

3. Ergot on the arterioles of the frog's web. Wrap a frog in a wet cloth and fasten to a frog-board for examining the web. Give a lymph sac injection of **0.5 c.c. fluid extract of ergot.** Select a good field of small arterioles and measure their diameter at once. The relative change in diameter of small vessels can be determined by selecting a field in which pigment spots mark the borders of the vessels. Sketch such a vessel and spots for the normal. Re-sketch after the drug. Re-measure at intervals of five minutes as the ergot is absorbed.

4. Ergot on the blood-pressure of a mammal. Give an intravenous dose of **0.5 c.c. fluid extract** of **ergot** to a mammal while taking a record of the blood-pressure.

SUPRARENAL GLAND.

The commercial preparation of the active principle of suprarenal gland, adrenalin hydrochloride, presents the same physiological action as the gland extract and has the special advantage of preparation in definite and known strengths. It has come into general use for therapeutic purposes and is, therefore, used in these experiments.

Experiments Showing the Action of Adrenalin Hydrochloride. PAGE.

1. On the frog 56
2. On the ventricular strip 56
3. On the frog's heart 56
4. On the isolated mammalian heart 56
5. On the simple muscle contraction 58
6. On muscle work 58
7. On the local mucous surfaces 58
8. On the size of the blood-vessels in the frog's web 58
9. On general blood-pressure and peripheral vaso-constriction 58

1. **Adrenalin on the frog.** Give 0.5 c.c. 0.1 percent in the dorsal lymph sac.

2. **Adrenalin on the ventricular muscle.** Transfer a terrapin's ventricular strip contracting in physiological saline to **0.01 percent adrenalin** in saline. Change after two to five minutes. The drum speed should be 1 cm. per minute. Suprarenal extract has also been shown to increase the amplitude and the rate of the ganglion free ventricular muscle of the dog. Cleghorn, Amer. Jour. Physiol., Volume III, p. 273.

3. **Adrenalin on the frog's heart.** Use the perfusion method, page 68, with the heart in place and the inflow canula in the ascending vena cava. Follow physiological Ringer perfusion with **0.001 percent adrenalin hydrochloride** in Ringer. The drum speed should be 2 mm. per second. Direct application to the surface of the heart requires a strength of at least 0.05 percent adrenalin hydrochloride.

4. **Adrenalin on the isolated heart.** Perfuse a cat's heart in the usual way with Locke-blood solution for a normal, then change to a **0.0001 percent adrenalin hydrochloride** in Locke-blood. If the heart be beating feebly it often happens that the contractions will increase in amplitude by 200 percent and more.

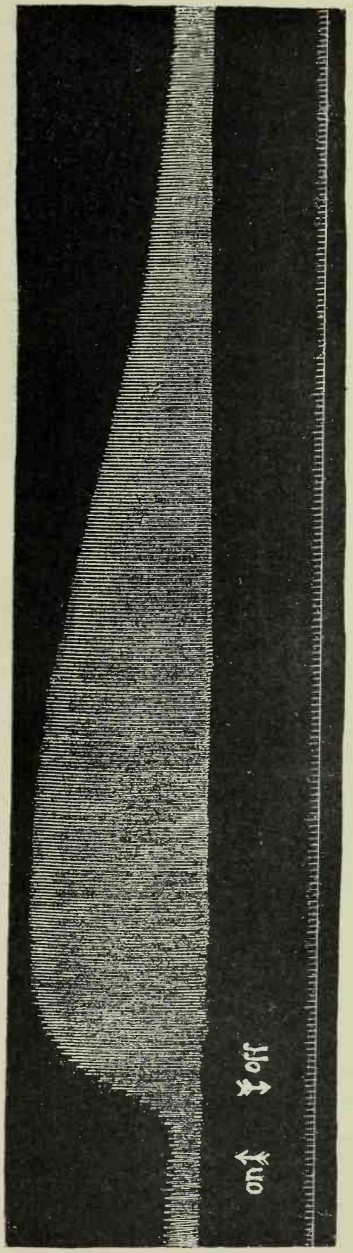

FIG. 31.—Action of 0.0001 percent adrenalin hydrochloride on the isolated cat's heart. The heart was giving weak contractions just before receiving the drug. Perfused between the arrows with adrenalin. Time in seconds.

5. Adrenalin on the simple muscle contraction. Ligate one leg of a frog and give **0.5 c.c. of 0.05 percent adrenalin.** Allow ten minutes for absorption. Compare the simple muscle contractions of the two gastrocnemii as repards **a,** amplitude, and **b,** the time of the simple contraction. The muscular power of patients with Addison's disease has also been shown to be greatly improved by giving the extract of suprarenal gland.

6. Adrenalin on muscle work in the frog. Prepare a frog as in experiment 5 above and test the work performed by the two muscles. For details of procedure see alcohol experiment 5.

7. Adrenalin on mucous surfaces. Paint one-half the tongue with **0.1 percent adrenalin hydrochloride.** At intervals of five minutes drop **0.01 percent** in saline (sterilize by boiling) in one eye. Compare the two halves of the tongue and the two eyes as to vascular condition. Examine the size of the pupils. Test for possible differences as to the sensitiveness of the conjunctiva. Try the effect in the eye of a cat or dog.

8. Adrenalin on the size of the blood-vessels of the frog's web. Use a dose of **0.5 c.c. of 0.1 percent** as a hypodermic. See ergot experiment 3, page 55; nitroglycerine experiment 3, page 59. Or apply drops of **0.1 percent** directly to the web under the microscope.

9. Adrenalin on general blood-pressure and on vaso-constriction in a mammal. Prepare a dog for blood-pressure. Adjust an onkometer to the left kidney and record the change in volume with a Brodie's bellows or Roy's piston recorder. Give **2 to 4 c.c.** of **0.01 percent adrenalin hydrochloride** slowly in a vein. Give **2 c.c. of 0.1 percent atropine** to eliminate the vagus action on the heart and repeat the adrenalin. Compare with digitalis, page 53; ergot, page 55; veratrine, page 50. Drugs of antagonistic action are nitrites and potash salts.

NITROGLYCERINE AND THE NITRITES.

Experiments on the Action of Nitroglycerine and the Nitrites.

PAGE.
1. On the frog 59
2. On the heart muscle 59
3. On the arterioles of the frog 59
4. On the circulation volume 59
5. Amyl nitrite on the pulse 59
6. Nitrites on mammalian blood-pressure 60

Nitroglycerine and the nitrites affect primarily the peripheral circulation, causing vaso-dilation with fall of blood-pressure. The specific action is on the muscular tissue.

1. Nitroglycerine on the frog. Give a frog a dose of **0.5 c.c. of 0.1 percent nitroglycerine** in the dorsal lymph sac.

2. Sodium nitrite on the heart muscle. Test the action of **0.02 percent sodium nitrite** on the contracting ventricular strip.

3. Nitroglycerine on the arterioles of a frog. Bind a frog for the microscopic examination of the web circulation. Then give **1 c.c. of 0.1 percent nitroglycerine** in the lymph sac. Immediately measure the smaller arterioles in a favorable field and re-examine every two minutes as absorption progresses. Try direct application of drops of 0.1 percent to the web.

4. Sodium nitrite on the circulation volume. Pith a frog or small terrapin. Insert a canula in the aorta or one of its branches, snip the veins with the scissors to allow free perfusion, set the frog-board at an angle to facilitate drainage of liquid. Perfuse the blood-vessels with a weaker Ringer's solution for a normal. Follow with **0.01 percent sodium nitrite** in the weaker Ringer, keeping a uniform pressure of the perfusion liquids of from 6 to 10 cm. Measure the perfusion rate in drops per minute, or collect the outflow in a 25 c.c. graduate.

Test the amount of outflow when irrigated with 0.0005 percent of soluble digitalis, then follow with 0.0001 percent sodium nitrite, both in the weaker Ringer.

5. Amyl nitrite on the pulse. Take normal pulse records with one of the standard sphygmographs. Break an amyl nitrite pearl on a handkerchief and breathe deeply the fumes. Pulse tracings taken 5 and 10 minutes later will show the usual signs of dilated blood-vessels with accompanying low pressure. Slight headaches sometimes follow the use of amyl nitrite.

6. Nitrites on mammalian blood-pressure. Anesthetize a 10 kilo dog and take the blood-pressure. Give intravenous doses of nitrites in the following order, repeating with larger doses if necessary and always allowing full time for recovery: **1 c.c. of 0.1 percent nitroglycerine, 3 c.c. of 0.1 percent; 2 c.c. of 0.1 percent amyl nitrite; 6 c.c. of 0.1 percent**

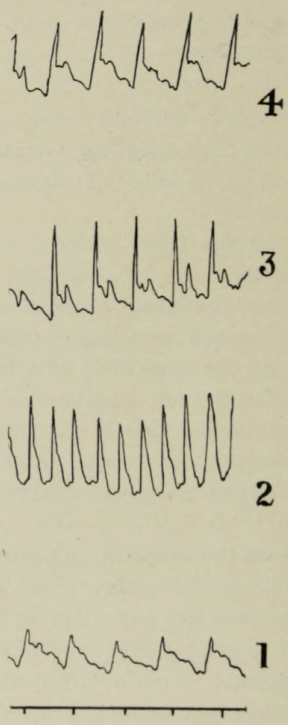

FIG. 32.—Action of amyl nitrite on the human pulse. One amyl nitrite pearl was crushed on a handkerchief and the fumes inhaled deeply. Trace 1 is the normal pulse. Trace 2 immediately after amyl nitrite fumes. Traces 3 and 4 are stages of recovery. Time in seconds.

sodium nitrite. The blood-pressure remains low for a long time after sodium nitrite. A dose of **2 c.c. to 5 c.c. of 0.2 percent digitalis** or **2 c.c. of 0.01 percent adrenalin hydrochloride** will antagonize this effect.

Give **2 c.c. of 0.1 percent atropine** to eliminate the action of the cardiac nervous apparatus and repeat the above doses of nitroglycerine and sodium nitrite.

CARBOLIC ACID.

Experiments on the Action of Carbolic Acid.

PAGE.
1. On the frog. 61
2. On the growth of yeast and bacteria. 61
3. On the circulatory and respiratory systems of a mammal 61

1. Carbolic acid on the frog. Give a dose of **1 c.c. of 1 percent.**

2. Carbolic acid on the growth of yeast and of bacteria. Prepare six fermentation tubes of active yeast culture and as many test-tubes of inoculated bouillon. Keep one tube of each for a normal and to the others add enough **10 percent carbolic acid** to make a series of 0.1, 0.5, 1, 2, and 4 percent solutions. Keep at laboratory temperature and observe through a period of several days.

3. Carbolic acid on the circulatory and respiratory systems of the mammal. While taking records of blood-pressure and respiration by the usual method give an intravenous injection of **10 c.c. of 0.5 percent carbolic acid.** When the collapse stage is far advanced inject **1 percent sodium sulphate** slowly. Judge the amount required by the action in overcoming the carbolic acid depression of the respiratory apparatus.

POTASSIUM SALTS.

Experiments Showing the Action of Potassium Salts.

PAGE.
1. On the heart muscle 61
2. On the reaction time in the reflex frog. 61
3. On muscle irritability and muscle work in the frog. . . . 61

1. Potassium chloride on the heart muscle. A ventricular strip contracting in physiological saline solution is transferred to **0.06 percent potassium chloride** in saline for two to five minutes. Contractions return in saline even after stronger doses of potash.

2. Potassium bromide on the reaction time in the reflex frog. Compare the reaction time of a reflex frog before and 20 to 40 minutes after **0.3 c.c. of 5 percent potassium bromide** in the dorsal lymph sac.

3. Potassium chloride on muscle irritability and muscle work in the frog. Compare the two gastrocnemii as to irritability and as to amount of muscular work done. Dose **0.3 c.c. of 5 percent** solution given hypodermic after one leg is ligatured.

CALCIUM SALTS.

Experiments Illustrating the Action of Calcium Salts. PAGE.

1. On heart muscle 62
2. On the frog's heart 62
3. On the blood-pressure and the respiration in the mammal. 63

1. **Calcium chloride on heart muscle.** Transfer a ventricular strip from physiological saline to **0.03 percent calcium chloride** in saline for three to five minutes. Record on a drum speed of 2 cm. per minute. Repeat, using **0.06 percent.** The rate is increased and the amplitude often

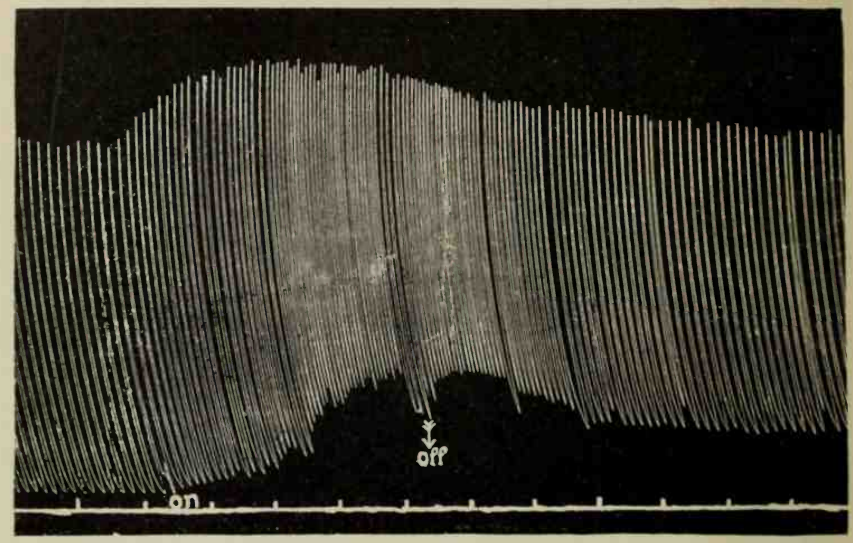

FIG. 33.—Terrapin's heart muscle as influenced by a solution of 0.06 percent calcium chloride in physiological saline. A weaker solution produces much less tone. Time in seconds.

doubled. The stronger solution produces great increase in tone which sometimes passes into delirium cordis. Potash salts antagonize. Read Ringer, Jour. Physiology, 1883.

2. **Calcium chloride on the frog's heart.** Perfuse the frog's heart through the vena cava with 0.7 percent sodium chloride and follow with **0.03 percent calcium chloride** in 0.7 percent sodium chloride. Recover

the sodium chloride type of contractions, then perfuse with 0.01 percent barium in sodium chloride.

3. Calcium chloride on the blood-pressure and the respiration in the mammal. Give an intravenous dose of **10 c.c. of 1 percent** for a dog. Cut the vagi and repeat the dose. Alternate the dose with potassium, **2 to 4 c.c. of 1 percent.**

BARIUM SALTS.

Experiments on the Action of Barium Salts.

PAGE.
1. On the frog 63
2. On the heart muscle 63
3. On the circulation and on the respiration movements in mammals 63

1. Barium on the frog. Dose, **1 c.c. of 1 percent barium chloride.**

2. Barium chloride on the heart muscle. Transfer a contracting ventricular strip from 0.7 percent sodium chloride to **0.01 percent barium chloride** in saline. Short immersions increase the rate, but long baths show that this salt does not sustain contractions as do calcium salts. A 0.1 percent solution in saline delays contractions with prevention of relaxation. Contractions still take place in 1 percent barium chloride. Compare with digitalis.

3. Barium chloride on the circulation and on respiratory movements in mammals. The effect on the heart and blood-pressure and on respiration in a mammal is demonstrated by an intravenous dose of **5 c.c. of 0.2 percent** given slowly. This dose should be repeated several times both before and after section of the vagi. Barium salts act as strong poisons to the nerve centers, especially those in the medulla.

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OPERATIONS, APPARATUS AND SPECIAL METHODS.

PHYSIOLOGICAL SOLUTIONS.

The lymph and blood plasma in which the tissues develop are the true physiological solutions.

Artificial solutions imitate lymph in its isotonicity—its physical character, and in its composition—its chemical character. Sodium chloride in 0.6 percent solution, used first by Nasse in 1869 on frog's muscle, and by Bowditch in 1871 on the frog's heart, was supposed to prevent injurious changes in the tissue by virtue of its isotonicity. Ringer in 1883 and Locke in 1885 introduced the solutions which bear their names. They showed that the chemical factors play a fundamental part in the effects of these solutions on the tissues. At the present time we recognize that exact isotonicity is not nearly so fundamental as at first supposed, and that these solutions are chemically active in relation to the living protoplasm.

At the present time much attention is being given to the effects of asphyxiation on the physiological activity of living tissues that are isolated from the normal circulation. The artificial solutions can be made more efficient by shaking with air before using, or by shaking with pure oxygen. Isolated mammalian hearts give much more constant characters in their response to artificial solutions which contain defibrinated blood, preferably from the animal supplying the heart. Even with five to ten percent of blood such solutions are strikingly more efficient, supposedly because they are much better oxygen carriers. For cats' hearts Locke-blood solutions aerated by a stream of oxygen are very efficient indeed.

1. **Physiological salt solution or normal saline.** Sodium chloride in distilled water 0.7 percent. More exact isotonicity is secured by 0.6 percent for frogs, 0.7 percent for terrapin and 0.9 percent for mammals.

2. **Ringer's solution.** The Ringer's solution that imitates the blood serum in its effects on heart tissue is made up in this laboratory in the following proportions:

Sodium chloride, 0.7 percent.
Potassium chloride, 0.03 percent.

Calcium chloride (cryst. computed water free), 0.026 percent.

For heart work where a more rapid rate is desired the amount of potassium must be reduced to that in Ringer's original formula.

Sodium chloride, 0.7 percent.

Potassium chloride, 0.01 percent.

Calcium chloride (cryst. computed water free), 0.026 percent.

3. Locke's solution. Locke's solution is a mixture of the salts in Ringer's solution with dextrose added to make 0.1 percent.

Sodium chloride, 0.7 percent.

Potassium chloride, 0.03 (or 0.01) percent.

Calcium chloride (cryst), 0.026 percent.

Dextrose, 0.1 percent.

4. Locke-blood solution. Add 5 to 10 percent of defibrinated whole blood to the above Locke's solution.

ANESTHESIA.

The mammals usually available for laboratory experimental purposes are dogs, cats, rabbits and guinea-pigs, each of which can best be anesthetized by a special treatment of its own.

Dogs. Give a 10-kilo dog **1 c.c.** (17 minims) **of 2 percent morphine** under the skin of the shoulder, holding its head firmly between the operator's legs while the hypodermic injection is being given. Allow 15 minutes or more for the morphine to take effect. The morphine should be followed by **chloroform** or **chloroform and ether** in equal parts. Give it by means of a small nose hood made by sewing a cheese-cloth, that has been folded in the form of a blunt cone, to a wire ring, or use a Senn's inhaler mask. When the voluntary movements have about ceased, tie the dog to a holder and take it to the experimental table. The tests of good anesthesia are: 1, loss of voluntary movements; 2, no cutaneous reflexes; 3, slight corneal reflexes or none in deep anesthesia; 4, even and fairly deep respiration; 5, medium blood-pressure and pulse. This condition of **anesthesia is maintained by giving chloroform from a dropping-bottle at absolutely regular intervals of 30 seconds by the watch.** The number of drops necessary for each animal will quickly be found by trial. In the experience of this laboratory it is from 3 to 6 drops per 30 seconds. The success of most pharmacological experiments on dogs depends upon maintaining an absolutely even anesthesia.

Cats. A mixture of equal parts of **chloroform and ether** is the most

practical anesthetics for cats. These animals are anesthetized most conveniently by putting them in a box of about two cubic feet in dimension and provided with a close cover. A very convenient box is the tin display cracker box with glass window obtained of the grocer. Drop in the box with the cat a small strip of cheese-cloth saturated with chloroform-ether mixture, 10 c.c. in broken doses will anesthetize a cat in 10 minutes. As soon as the animal falls down under the influence of the anesthetic it should be taken from the box, fixed in the holder, and the anesthetic given from a cloth in the manner and with care prescribed above for the dog. Cats do not survive pure chloroform in the hands of the ordinary student anesthetist.

Rabbits. Give rabbits **2 grams of urethane** by the mouth. Follow with light and careful use of **ether.** Or pure ether may be given without the urethane. Give the ether in the manner and with the regularity recommended above for giving chloroform to dogs. Do not use chloroform or even chloroform mixtures with rabbits.

Guinea-pigs. These little animals when they must be used for pharmacological purposes are anesthetized best with pure ether or ether followed with a little morphine.

THE PREPARATION OF THE VENTRICULAR MUSCLE.

Destroy the brain of a terrapin, remove the plastron and open the pericardium. Grasp the left angle of the base of the exposed ventricle with a forceps and cut with a scissors from this point around the apex to the opposite side, thus removing a piece about one inch long and the size of the half of a small lead pencil. Split this strip into two or three smaller ones for class use.

To mount the heart strip tie silk threads to each end, one with a loop one-half inch long and the other with a loop about four inches long. Place the short loop on the hook of the glass-rod support provided for the purpose, and the long loop over the recording lever. Use a straw lever of the powerfulcrum-weight order mounted in a muscle lever holder. A total tension of one gram is best for developing the contractions of the ventricular strip.

The holder mentioned above is made of a glass rod 4 to 5 mm. diameter and 15 cm. long. Bend it at a right angle in the middle and then draw out and turn a hook on one end, the hook being turned back on the rod. The apparatus set up complete consists of a single iron stand with three clamps, the top one to support the lever holder, the middle the glass rod, and the bot-

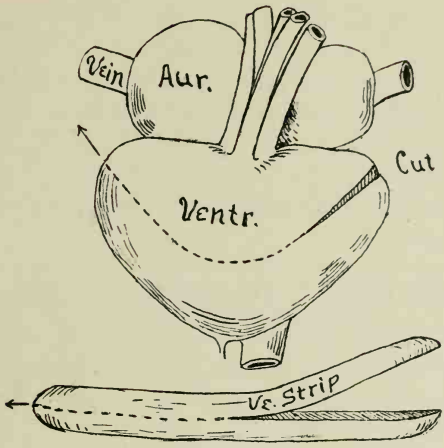

FIG. 34.—The terrapin's heart, ventral view, showing how to cut an apex strip for experimental purposes and how to split this apex into smaller pieces.

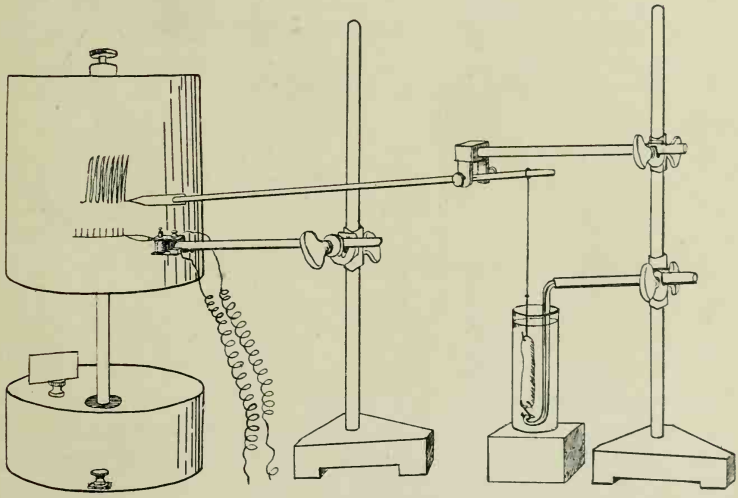

FIG. 35.—Apparatus as set up to demonstrate the contractions of the apex muscle of terrapin's ventricle. The glass L-shaped holder should be set on the stand high enough to allow of easy change of solution tubes. The figure shows the tube of physiological saline and other details for the better illustration of the mounting of the heart strip

tom one a platform on which rests the footed test-tube (1 x 3 inch specimen tube) to contain the solution surrounding the strip. The ventricular strip mounted in this apparatus with a tension of one gram and bathed in a solution of 0.7 percent sodium chloride will begin rhythmic contractions in from 10 to 40 minutes. These contractions will continue about two hours, growing constantly smaller for the entire time. The strip may then be revived by a bath of Ringer's solution or by serum, and may again be used in the sodium chloride bath.

TO TEST THE ACTION OF DRUGS ON THE FROG'S OR TERRAPIN'S HEART.

Two methods are used in this laboratory for the study of the action of drugs on the frog's heart, both permitting of permanent records. The

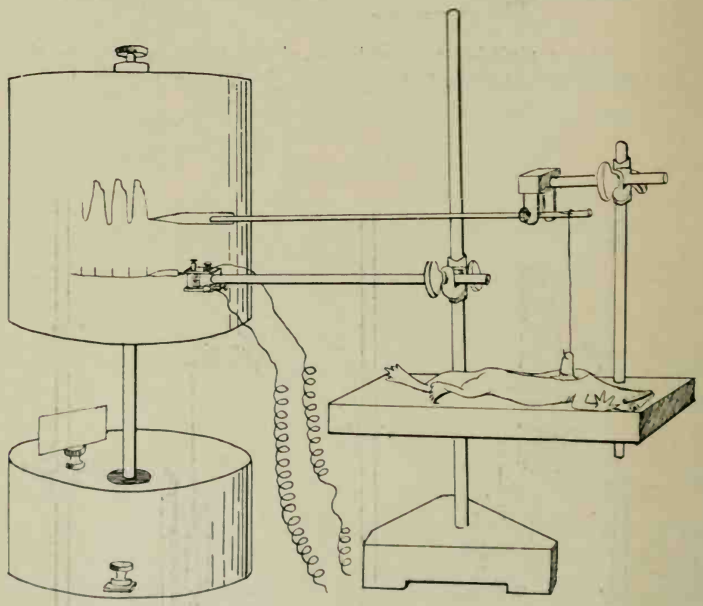

FIG. 36.—Showing the method of recording the action of the frog's heart in place in the body cavity. If the perfusion method is used the canula can be inserted with greater ease if the frog is reversed on the support.

most convenient method is to pith the frog, open the thorax and expose the heart, adjust the foot of a delicately poised heart lever on the venticle and, while the record is being taken, irrigate the surface of the heart with the

drug. Dissolve the drug in physiological saline and always take a previous normal record under saline irrigation. This method requires the use of relatively strong solutions. The most convenient irrigating bottles are four- or eight-ounce aspirator bottles with tubed foot for rubber connector. These are each provided with a small-mouthed canula attached by a short rubber connection, and the flow is regulated by a screw compress. Fit these flasks with Marriotte stoppers and support them on a stand by a universal buret clamp about the neck.

The second method, that of perfusion, is carried out best as described by Walden in the American Journal of Physiology, Volume III, page 123. Insert a canula into the inferior vena cava for an inflow and one in the aorta for an outflow, or merely cut one aortic branch and let the outflow go free. The canula is connected with two supply bottles, one for physiological saline, the other for the drug in solution. The Marriotte stoppers should be set at exactly the same pressure levels. Connect the two flasks with the inflow canula by a T-tube brought as close as possible to the heart in order that the solutions may be changed quickly with only a short connecting tube to be washed out. Very weak solutions of drugs are required by this method of perfusion. The frog's heart is quickly exhausted in pure saline solutions, so, for certain prolonged experiments it is better to use the weaker Ringer's solution for dissolving the drug.

Record the contractions of the ventricle by a thread from its apex to the ventrical arm on a balanced horizontal lever. A flexible paper or celluloid writing point will add to the accuracy and beauty of the records. These writing points should be 2 to 3 cm. long by 0.5 cm. wide and made of light-weight but hard note paper.

IRRIGATING AND PERFUSING FLASKS.

The quarter- and half-pint aspirator bottles manufactured by Whitall, Tatum & Co., with tubed foot for attaching a rubber tube are particularly adapted to both irrigation and perfusion of the heart. For use in irrigation these bottles are clamped to a heavy based stand by a universal buret clamp on the neck. Insert a tight-fitting rubber stopper with a 2 mm. glass tube, to give a constant pressure level. A short heavy rubber connector provided with a small screw compress and a glass dropper serves to regulate the speed of the outflow. Such a flask attached to an independent stand and set at a level so that the fluid drops only a few millimeters is an exceptionally satisfactory method of applying solutions directly to the surface of the heart.

Two perfusion bottles may be connected together by a T-tube for perfusion work. In this case the inflow canula is connected by a very short (6 to 10 cm.) tube of small caliber and is supported firmly by a clamp on the T-tube. Or a Y-canula can be used and the flasks attached directly to its limbs. In either case it is better to insert a small T-tube with light spring clamp in order to wash out any drugs in the tubing. This canula, when provided with an overflow, as shown by Gibson and Schultz in an article now in manuscript, permits change from one solution to another without a break in the pressure of the fluids. The connecting tubes for the flasks should be 25 to 30 cm. long to permit adjusting. Set screw or spring compresses near the bottle. Fill one flask with the normal solution, the other with the drug. A very small amount of the fluid can be applied by means of these perfusion flasks.

TO TEST THE ACTION OF DRUGS ON THE BLOOD-PRESSURE, RESPIRATION, ETC., OF A MAMMAL.

1. **The anesthetic.** For anesthesia methods see page 65.

2. **The operations.** Blood-pressure is taken from one of two arteries, the right common carotid, or the femoral artery. The femoral is practical only for the dog. To expose the **common carotid** make a three-inch cut over the trachea from the proximity of the larynx to the manubrium. Separate the muscles down to the trachea, and then along the side of the trachea till the common carotid artery and vagus come into view. Use the scalpel handle and tear rather than cut the facias and muscles involved. Avoid the veins and the laryngeal arteries. No blood need be lost after the skin is cut. Separate the fascia binding the artery and vagus, using care not to injure the latter. Place a bulldog forceps on the artery well toward the thorax. Ligate the cephalic end. Lay and tie a ligature loosely about the intervening stretch of artery for the canula. Grasp the artery at the cephalic ligature and use the tip of the scissors to make a V-shaped cut two-thirds through the artery wall and directed toward the heart. Insert the canula and ligate it firmly with the ligature already laid.

The **femoral** artery is exposed by a 5 cm. cut over the artery where the pulse can be felt near Poupart's ligament. The artery is prepared and the canula inserted as described for the carotid.

The **saphenous** vein or the **jugular** are used for injecting drugs. Insert a small washout canula toward the heart choosing the vein exposed by the previous operation. Keep the vein closed with a bulldog forceps in

order to prevent small clots in the mouth of the canula, except when injections are to be made.

Tracheotomy should generally be performed for all student work on the mammals used in blood-pressure experiments in pharmacology. Free the trachea immediately below the thyroid cartilage and insert a metal canula made especially for the dog, or insert one limb of a glass T-tube of as large size as the trachea will take. Tie it firmly with small stout twine.

The apparatus consists of a continuous paper kymograph (Ludwig's weight-driven pattern arranged to run the paper in the right-handed direction is the most satisfactory instrument); mercury manometer for measuring the blood-pressure; respiration tambour (Marey's form); signal pen to record stimulations, injections and other events; time signal; stimulating coil and accessories complete; and a jacketed buret for transfusing warm solutions into the vein. The recording pens of the manometer, tambour, signals, etc., must all be adjusted to the kymograph in an exact vertical line. Fill the lead tube of the manometer with 10 percent magnesium sulphate from a pressure bottle, take the zero level of the manometer, set the time signal to write on this level, connect with the canula, and fill to a pressure of 130 mm. mercury. Connect the respiration tambour directly with the side branch of the tracheal tube. Start the kymograph, ink all the pens, remove the arterial bulldog clamp, and the experiment is ready to begin.

A renal onkometric record should be taken with blood-pressure in the investigations on certain drugs. Open the abdomen along the entire median line, cut the wall transversely for two to three inches over the left kidney. Strip the kidney of its fat and looser coverings and enclose it in a renal onkometer. Adjust the overflow from the onkometer to the exact kidney level and take a record of the variations with a small sized Brodie's bellows recorder adjusted in line with the recorders mentioned above.

METHOD OF TESTING THE ACTION OF DRUGS ON THE REFLEXES OF A FROG.

Carefully destroy the brain and the medulla, but not the cord. Prevent the loss of blood. Suspend the frog to a horizontal rod on a stand, using a card hanger or a loop of string on the upper jaw. Stimulate the tip of the toe with acid or with platinum electrodes and measure the reaction time by counting seconds until the foot is withdrawn. The reaction time may be recorded on a kymograph. Attach a horizontal writing point of mucilaged paper to the leg above the foot. Take the speed of the drum with

one magnet beating seconds, and record the instant of stimulation with a second and independent magnet controlled by a contact key.

Take the normal reaction time first, then give the drug as an injection in the dorsal lymph sac and allow about 20 minutes for absorption. Remeasure the reaction time and repeat at intervals of 10 minutes to get the progressive effects of the drug.

METHOD OF GIVING AND TESTING THE ACTION OF A DRUG ON THE FROG'S GASTROCNEMIUS MUSCLE.

One should always compare the drugged muscle with a normal or undrugged muscle from the same frog. This may be done in one of two ways. 1st. With the circulation undisturbed. Pin the frog face down on a frog-board, isolate and attach the tendon Achilles to a muscle lever, isolate and stimulate the sciatic at its origin in the lumbar plexus using care not to disturb the circulation, or stimulate the muscle directly. After a normal record is secured then give the drug in the usual way and take a record of the other or drugged muscle. 2d. Lay a ligature about one leg near the thigh tight enough to stop its circulation. Give the drug by injection into the dorsal lymph sac, or abdominal cavity, and after absorption is complete and the tissues have been acted on by the drug (20 to 30 minutes) dissect out the gastrocnemii and test. Always use the undrugged muscle first and the drugged one immediately following. While the normal muscle work is being tested the drugged leg should have its circulation stopped by ligature. This leaves the two muscles in more nearly the same state of nutrition and asphyxiation. It is usually best to stimulate the muscle directly. There are three tests that can be applied: 1, Irritability, by the minimal stimulus method; 2, Rapidity of the simple muscle contraction; 3, The amount of work a muscle will do with simple contractions at constantly repeated intervals. In this latter test stimulate once in two seconds, record on a drum with speed of 1 mm. per second.

TRANSFUSION BURET FOR MAMMALS.

Transfusions of several cubic centimeters of liquid should be warmed to body temperature. Inclose a 50 c.c. buret in an ordinary Liebig's condenser jacket and mount vertically on a heavy base stand. Mount and connect a 6-inch funnel with the upper side tube of the condenser. Attach a rubber tube fitted with a spring compress clamp on the lower side tube to

regulate the outflow of the warm water introduced by the funnel to keep the perfusion liquid at the proper temperature. Mount a thermometer inside the condenser with its bulb near the lower end of the apparatus. The buret connections with the transfusion canula should be as short as possible and their tubes should be provided with light screw-compresses. Where only 1 or 2 c.c. of liquid is to be introduced it is unnecessary to warm it. In fact a hypodermic syringe is most convenient where the volume of the injection does not exceed 1.5 c.c.

APPARATUS FOR THE STUDY OF THE ISOLATED MAMMALIAN HEART.

The mammalian heart isolated completely from the body can be maintained in constant activity for several hours. It gives constant responses to drugs in solution in the perfusion liquid best adapted to maintain its life, i.e., Locke's solution with a small quantity of the animal's defibrinated blood. Cats and rabbits are especially well adapted to this experiment. The smaller size of the cat's or rabbit's heart makes it preferable to that of a dog.

The points to be secured in the isolated heart apparatus are: 1. A uniform temperature of about 37° Centigrade. 2. An adjustable pressure for the perfusion fluid. 3. A device for quickly shifting from the normal perfusion to the drugged perfusion fluid without change in temperature, pressure or any other factor than the presence of the drug. 4. An accurate recording device.

The apparatus shown assembled in Fig. 37 accomplishes all of the above points. The gas water heater connected as shown will maintain a uniform temperature in the water jacket through which the perfusion tubes run to the heart canula. The overflow from the water jacket is conducted into a pan in which the perfusion fluid reservoirs receive preliminary warming. The heart is attached to a very short canula beneath the warming jacket and the overflow of perfusion fluid maintains a temperature of the heart only slightly below that of the warming jacket.

The pressure on the heart, i.e., on the perfusion fluid, is accomplished by connecting the perfusion bottle with an air or oxygen reservoir, and this in turn with a water reservoir which can be raised or lowered. The flow of water from the pressure bottle into the closed system produces the desired pressure on the perfusion system. At the same time the perfusion fluids are aerated by the air (or oxygen) as it is forced into the reservoir, a result

accomplished by conducting the perfusion bottle inlet tubes to the bottom of the containers.

A uniform pressure is secured on both the normal and the drugged perfusion fluids by the system of tubes shown. If the clamp is removed from

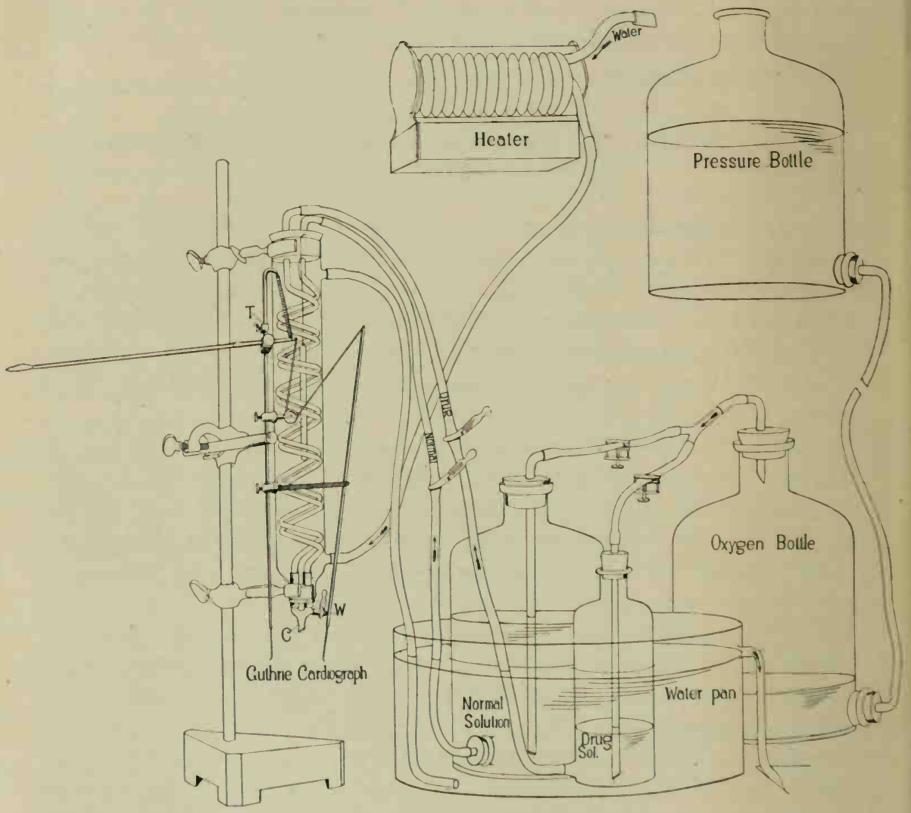

FIG. 37.—Illustrating the assembly of apparatus for the pharmacological study of the isolated heart of a mammal. The legends on the apparatus are self-explanatory.

the outflow tube of the drugged perfusion fluid at the exact moment a second clamp is placed on the tube from the normal fluid reservoir (or *vice versa*), the shift will be accomplished without change of pressure on the heart. The tubes run independently to the canula which is itself so short that the time

from the moment of turning a perfusion fluid on or off is reduced to a minimum. The canula is provided with a side washout tube.

The Guthrie cardiograph shown is very adjustable in all essential features. It gives satisfactory and accurate records, if care is used in inserting the lever tips into the walls of the heart. This apparatus permits a direct record on the ordinary kymograph. It also permits one to surround the heart with a warm cup or jacket where greater constancy of temperature is desired, as in research work.

LIST OF STOCK SOLUTIONS.

Make the solution up in 0.7 percent sodium chloride solution and in Ringer's solution. Special solutions must be prepared for the mammalian heart experiments.

Aconite 0.1 percent.
Adrenalin hydrochloride 0.001 percent, 0.01 percent, 0.05 percent, 0.1 percent.
Alcohol 95 percent, 2 percent, 5 percent, 10 percent, 20 percent.
Amyl nitrite 0.1 percent, pearls.
Atropine 0.001 percent, 0.002 percent, 0.1 percent, 0.2 percent, 1 percent, 2 percent, 5 percent, and 1/120 grain tablets.
Barium chloride 0.01 percent, 0.1 percent, 0.2 percent, 1 percent.
Caffeine 0.1 percent, 0.2 percent, 0.5 percent, 1 percent.
Calcium chloride 0.03 percent, 0.06 percent, 1 percent.
Carbolic acid 0.5 percent, 1 percent, 10 percent.
Chloral hydrate 0.1 percent, 1 percent, 2 percent.
Chloroform 0.05 percent, 0.1 percent, 0.5 percent, 20 percent in oil, pure.
Cocaine hydrochlorate 0.01 percent, 0.2 percent, 0.5 percent, 1 percent, 2 percent.
Codeine 0.5 percent, 1 percent.
Curare 0.2 percent, 1 percent.
Digitalis 0.0005 percent, 0.001 percent, 0.002 percent, 0.1 percent, 0.2 percent, 0.5 percent, 1 percent.
Ether 1 percent, 2 percent, 4 percent, 6 percent, 8 percent, pure.
Ergot Squibb's fluid extract, 10 percent of fluid extract.
Hyoscyamine 1 percent.
Locke's solution.
Morphine acetate 1 percent, 2 percent, 10 percent.
Nicotine 0.02 percent, 0.1 percent, 0.2 percent, 1 percent.
Nitroglycerine 0.1 percent.
Physiological saline 0.7 percent.
Physostigmine 0.1 percent, 1 percent.
Pilocarpine nitrate 0.1 percent, 1 percent, 10 percent.
Potassium chloride 0.03 percent, 1 percent, 5 percent.
Potassium bromide 5 percent.
Quinine hydrochlorate 0.1 percent, 1 percent.
Ringer's solution, weak, strong.
Sodium nitrate 0.01 percent, 0.02 percent.
Sodium sulphate 1 percent.
Strychnine nitrate 1 percent, 0.1 percent, .02 percent.
Thebaine 0.5 percent, 1 percent.
Veratrine 0.05 percent, 0.1 percent, 1 percent, 1 percent of fluid extract.

REPORT FORM FOR EXPERIMENTS ON FROGS.

Animal ..... Name .....

Weight ..... Date .....

Experiment .....

I. Normal Reactions.

1. Position, Activity, etc.	2. Circulation, Heart, Skin, Mucous Mem.	3. Respiration	4. Reflexes	5. Eye

II. Action of the Drug.

Time ..... Dose ..... How given ..... Dose per kilo. .....

Observations on:

h. m .s.	1. Position and Activities	2. Circulation	3. Respiration	4. Reflexes, etc.	5. Eye

III. Summary.

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